

WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7: C12N 15/31, C07K 14/295, C12N 1/21, 1/19, 5/10, Ć07K 19/00, C12N 15/62, C07K 16/02, A61K 39/118, 31/70, G01N 33/569, C12Q 1/68, A61K 48/00

(11) International Publication Number:

WO 00/34483

(43) International Publication Date:

15 June 2000 (15.06.00)

(21) International Application Number:

PCT/US99/29012

A2

(22) International Filing Date:

8 December 1999 (08.12.99)

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(30) Priority Data:

09/208,277 8 December 1998 (08.12.98) US 09/288,594 8 April 1999 (08.04.99) US 1 October 1999 (01.10.99) 09/410,568 US 09/426,571 22 October 1999 (22.10.99) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIALINFECTION

(57) Abstract

Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a Chlamydia antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

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COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION

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TECHNIGAL FIELD

The present invention relates generally to the detection and treatment of Chlamydial infection. In particular, the invention is related to polypeptides comprising a *Chlamydia* antigen and the use of such polypeptides for the serodiagnosis and treatment of Chlamydial infection.

BACKGROUND OF THE INVENTION

Chlamydiae are intracellular bacterial pathogens that are responsible for a wide variety of important human and animal infections. *Chlamydia trachomatis* is one of the most common causes of sexually transmitted diseases and can lead to pelvic inflammatory disease (PID), resulting in tubal obstruction and infertility. *Chlamydia trachomatis* may also play a role in male infertility. In 1990, the cost of treating PID in the US was estimated to be \$4 billion. Trachoma, due to ocular infection with *Chlamydia trachomatis*, is the leading cause of preventable blindness worldwide. *Chlamydia pneumonia* is a major cause of acute respiratory tract infections in humans and is also believed to play a role in the pathogenesis of atherosclerosis and, in particular, coronary heart disease. Individuals with a high titer of antibodies to *Chlamydia pneumonia* have been shown to be at least twice as likely to suffer from coronary heart disease as seronegative individuals. Chlamydial infections thus constitute a significant health problem both in the US and worldwide.

Chlamydial infection is often asymptomatic. For example, by the time a woman seeks medical attention for PID, irreversible damage may have already occurred resulting in infertility. There thus remains a need in the art for improved vaccines and pharmaceutical

compositions for the prevention and treatment of *Chlamydia* infections. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for the diagnosis and therapy of *Chlamydia* infection. In one aspect, the present invention provides polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, or a variant of such an antigen. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments,, the polypeptide comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of (a) a sequence of SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290; (b) the complements of said sequences; and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions. In specific embodiments, the polypeptides of the present invention comprise at least a portion of a *Chlamydial* protein that includes an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 5-14, 17-20, 26, 28, 30-32, 34, 39-43, 65, 89-109, 138-158, 167, 168, 224-262, 246, 247, 254-256, 292, and variants thereof.

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a *Chlamydial* protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

In a related aspect, polynucleotide sequences encoding the above polypeptides, recombinant expression vectors comprising one or more of these polynucleotide sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides fusion proteins comprising an inventive polypeptide, or, alternatively, an inventive polypeptide and a known *Chlamydia* antigen, as well as polynucleotides encoding such fusion proteins, in combination with a physiologically acceptable carrier or immunostimulant for use as pharmaceutical compositions and vaccines thereof.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody, both polyclonal and monoclonal, or antigen-binding fragment thereof that specifically binds to a *Chlamydial* protein; and (b) a physiologically acceptable carrier. Within other aspects, the present invention provides pharmaceutical compositions that comprise one or more *Chlamydia* polypeptides disclosed herein, or a polynucleotide molecule encoding such a polypeptide, and a physiologically acceptable carrier. The invention also provides vaccines for prophylactic and therapeutic purposes comprising one or more of the disclosed polypeptides and an immunostimulant, as defined herein, together with vaccines comprising one or more polynucleotide sequences encoding such polypeptides and an immunostimulant.

In yet another aspect, methods are provided for inducing protective immunity in a patient, comprising administering to a patient an effective amount of one or more of the above pharmaceutical compositions or vaccines.

In yet a further aspect, methods for the treatment of Chlamydia infection in a patient are provided, the methods comprising obtaining peripheral blood mononuclear cells (PBMC) from the patient, incubating the PBMC with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated T cells and administering the incubated T cells to the patient. The present invention additionally provides methods for the treatment of Chlamydia infection that comprise incubating antigen presenting cells with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated antigen presenting cells and administering the incubated antigen presenting cells to the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient. In certain embodiments, the antigen presenting cells are selected from the group consisting of dendritic cells, macrophages, monocytes, Bcells, and fibroblasts. Compositions for the treatment of Chlamydia infection comprising T cells or antigen presenting cells that have been incubated with a polypeptide or polynucleotide of the present invention are also provided. Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, within other aspects, methods for

removing *Chlamydial*-infected cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a *Chlamydial* protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of Chlamydial infection in a patient, comprising administering to a patient a biological sample treated as described above. In further aspects of the subject invention, methods and diagnostic kits are provided for detecting Chlamydia infection in a patient. In one embodiment, the method comprises: (a) contacting a biological sample with at least one of the polypeptides or fusion proteins disclosed herein; and (b) detecting in the sample the presence of binding agents that bind to the polypeptide or fusion protein, thereby detecting Chlamydia infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. In one embodiment, the diagnostic kits comprise one or more of the polypeptides or fusion proteins disclosed herein in combination with a detection reagent. In yet another embodiment, the diagnostic kits comprise either a monoclonal antibody or a polyclonal antibody that binds with a polypeptide of the present invention.

The present invention also provides methods for detecting *Chlamydia* infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous nucleotides of a polynucleotide sequence peptide disclosed herein, or of a sequence that hybridizes thereto.

In a further aspect, the present invention provides a method for detecting *Chlamydia* infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe. In one embodiment, the oligonucleotide probe

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comprises at least about 15 contiguous nucleotides of a polynucleotide sequence disclosed herein, or a sequence that hybridizes thereto.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

SEQUENCE IDENTIFIERS

SEQ ID NO: 1 is the determined DNA sequence for the C. trachomatis clone

1-B1-66.

SEQ ID NO: 2 is the determined DNA sequence for the C. trachomatis clone

4-D7-28.

SEQ ID NO: 3 is the determined DNA sequence for the C. trachomatis clone

3-G3-10.

SEQ ID NO: 4 is the determined DNA sequence for the C. trachomatis clone

10-C10-31.

SEQ ID NO: 5 is the predicted amino acid sequence for 1-B1-66.

SEQ ID NO: 6 is the predicted amino acid sequence for 4-D7-28.

SEQ ID NO: 7 is a first predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 8 is a second predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 9 is a third predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 10 is a fourth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 11 is a fifth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 12 is the predicted amino acid sequence for 10-C10-31.

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SEQ ID NO: 13 is the amino acid sequence of the synthetic peptide 1-B1-

66/48-67.

SEQ ID NO: 14 is the amino acid sequence of the synthetic peptide 1-B1-

66/58-77.

SEQ ID NO: 15 is the determined DNA sequence for the *C. trachomatis* serovar LGV II clone 2C7-8

SEQ ID NO: 16 is the determined DNA sequence for a first putative open reading frame from C. trachomatis serovar D

SEQ ID NO: 17 is the predicted amino acid sequence encoded by the first putative open reading frame from *C. trachomatis* serovar D

SEQ ID NO: 18 is the amino acid sequence of the synthetic peptide CtC7.8-12

SEQ ID NO: 19 is the amino acid sequence of the synthetic peptide CtC7.8-13

SEQ ID NO: 20 is the predicted amino acid sequence encoded by a second putative open reading from *C. trachomatis* serovar D

SEQ ID NO: 21 is the determined DNA sequence for clone 4C9-18 from $\it C$. trachomatis LGV II

SEQ ID NO: 22 is the determined DNA sequence homologous to Lipoamide Dehydrogenase from *C. trachomatis* LGV II

SEQ ID NO: 23 is the determined DNA sequence homologous to Hypothetical protein from *C. trachomatis* LGV II

SEQ ID NO: 24 is the determined DNA sequence homologous to Ubiquinone Mehtyltransferase from *C. trachomatis* LGV II

SEQ ID NO: 25 is the determined DNA sequence for clone 4C9-18#2 BL21 pLysS from C. trachomatis LGV II

SEQ ID NO: 26 is the predicted amino acid sequence for 4C9-18#2 from $\it C$. trachomatis LGV II

SEQ ID NO: 27 is the determined DNA sequence for Cp-SWIB from C. pneumonia strain TWAR

SEQ ID NO: 28 is the predicted amino acid sequence for Cp-SWIB from C. pneumonia strain TWAR

SEQ ID NO: 29 is the determined DNA sequence for Cp-S13 from C. pneumonia strain TWAR

SEQ ID NO: 30 is the predicted amino acid sequence for Cp-S13 from $\it C$. $\it pneumonia$ strain TWAR

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SEQ ID NO: 31 is the amino acid sequence for a 10mer consensus peptide from CtC7.8-12 and CtC7.8-13

SEQ ID NO: 32 is the predicted amino acid sequence for clone 2C7-8 from C. trachomatis LGV II

SEQ ID NO: 33 is the determined DNA sequence of a clone from C. trachomatis serovar D which shows homology to clone 2C7-8

SEQ ID NO: 34 is the predicted amino acid sequence encoded by the sequence of SEQ ID NO: 33

SEQ ID NO: 35 is the DNA sequence for C.p. SWIB Nde (5' primer) from C. pneumonia

SEQ ID NO: 36 is the DNA sequence for C.p. SWIB EcoRI (3' primer) from C. pneumonia

SEQ ID NO: 37 is the DNA sequence for C.p. S13 Nde (5' primer) from C. pneumonia

SEQ ID NO: 38 is the DNA sequence for C.p. S13 EcoRI (3' primer) from C. pneumonia

SEQ ID NO: 39 is the amino acid sequence for CtSwib 52-67 peptide from C. trachomatis LGV II

SEQ ID NO: 40 is the amino acid sequence for CpSwib 53-68 peptide from C. pneumonia

SEQ ID NO: 41 is the amino acid sequence for HuSwib 288-302 peptide from Human SWI domain

SEQ ID NO: 42 is the amino acid sequence for CtSWI-T 822-837 peptide from the topoisomerase-SWIB fusion of *C. trachomatis*

SEQ ID NO: 43 is the amino acid sequence for CpSWI-T 828-842 peptide from the topoisomerase-SWIB fusion of *C. pneumonia*

SEQ ID NO: 44 is a first determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II clone</u> 19783.3,jen.seq(1>509)CTL2#11-3', representing the 3' end.

SEQ ID NO: 45 is a second determined DNA sequence for the *C. trachomatis* LGV II clone 19783.4,jen.seq(1>481)CTL2#11-5', representing the 5' end.

SEQ ID NO: 46 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II clone</u>19784CTL2_12consensus.seq(1>427)CTL2#12.

SEQ ID NO: 47 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19785.4,jen.seq(1>600)CTL2#16-5', representing the 5' end.

SEQ ID NO: 48 is a first determined DNA sequence for the <u>C. trachomatis</u>

<u>LGV II clone</u> 19786.3,jen.seq(1>600)CTL2#18-3', representing the 3' end.

SEQ ID NO: 49 is a second determined DNA sequence for the *C. trachomatis*<u>LGV II clone</u> 19786.4, jen.seq(1>600)CTL2#18-5', representing the 5' end.

SEQ ID NO: 50 is the determined DNA sequence for the C. trachomatis LGV II clone 19788CTL2_21consensus.seq(1>406)CTL2#21.

SEQ ID NO: 51 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19790CTL2_23consensus.seq(1>602)CTL2#23.

SEQ ID NO: 52 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II clone</u> 19791CTL2_24consensus.seq(1>145)CTL2#24.

SEQ ID NO: 53 is the determined DNA sequence for the C. trachomatis LGV II clone CTL2#4.

SEQ ID NO: 54 is the determined DNA sequence for the *C. trachomatis* LGV II clone CTL2#8b.

SEQ ID NO: 55 is the determined DNA sequence for the *C. trachomatis* LGV II clonel 5-G1-89, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 56 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II clone 14-H1-4</u>, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 57 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-G3-83, sharing homology to the hypothetical protein CT622.

SEQ ID NO: 58 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-B3-95, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 59 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-H4-28, sharing homology to the dnaK gene CT396.

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SEQ ID NO: 60 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II clone 11-H3-68</u>, sharing partial homology to the PGP6-D virulence protein and L1 ribosomal gene CT318.

SEQ ID NO: 61 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-G1-34, sharing partial homology to the malate dehydrogenase gene CT376 and to the glycogen hydrolase gene CT042.

SEQ ID NO: 62 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-G10-46, sharing homology to the hypothetical protein CT610.

SEQ ID NO: 63 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-C12-91, sharing homology to the OMP2 gene CT443.

SEQ ID NO: 64 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II clone 11-A3-93</u>, sharing homology to the HAD superfamily gene CT103.

SEQ ID NO: 65 is the determined amino acid sequence for the *C. trachomatis* LGV II clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 66 is the determined DNA sequence for the *C. trachomatis* LGV II clone CtL2#9.

SEQ ID NO: 67 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone CtL2#7.

SEQ ID NO: 68 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone CtL2#6.

SEQ ID NO: 69 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone CtL2#5.

SEQ ID NO: 70 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone CtL2#2.

SEQ ID NO: 71 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II</u> clone CtL2#1.

SEQ ID NO: 72 is a first determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 23509.2CtL2#3-5', representing the 5' end.

SEQ ID NO: 73 is a second determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 23509.1CtL2#3-3', representing the 3' end.

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SEQ ID NO: 74 is a first determined DNA sequence for the <u>C. trachomatis</u>

<u>LGV II</u> clone 22121.2CtL2#10-5', representing the 5' end.

SEQ ID NO: 75 is a second determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 22121.1CtL2#10-3', representing the 3' end.

SEQ ID NO: 76 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone 19787.6CtL2#19-5', representing the 5' end.

SEQ ID NO: 77 is the determined DNA sequence for the <u>C. pneumoniae LGV</u> II clone CpS13-His.

SEQ ID NO: 78 is the determined DNA sequence for the <u>C. pneumoniae LGV</u>

II clone Cp_SWIB-His.

SEQ ID NO: 79 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone 23-G7-68, sharing partial homology to the L11, L10 and L1 ribosomal protein.

SEQ ID NO: 80 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II</u> clone 22-F8-91, sharing homology to the pmpC gene.

SEQ ID NO: 81 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II</u> clone 21-E8-95, sharing homology to the CT610-CT613 genes.

SEQ ID NO: 82 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 19-F12-57, sharing homology to the CT858 and recA genes.

SEQ ID NO: 83 is the determined DNA sequence for the <u>C. trachomatis LGV</u> II clone 19-F12-53, sharing homology to the CT445 gene encoding glutamyl tRNA synthetase.

SEQ ID NO: 84 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II</u> clone 19-A5-54, sharing homology to the cryptic plasmid gene.

SEQ ID NO: 85 is the determined DNA sequence for the <u>C. trachomatis LGV</u>

<u>II</u> clone 17-E11-72, sharing partial homology to the OppC_2 and pmpD genes.

SEQ ID NO: 86 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 17-C1-77, sharing partial homology to the CT857 and CT858 open reading frames.

SEQ ID NO: 87 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u> clone 15-H2-76, sharing partial homology to the pmpD and SycE genes, and to the CT089 ORF.

SEQ ID NO: 88 is the determined DNA sequence for the <u>C. trachomatis LGV</u> <u>II</u>-clone 15-A3-26, sharing homology to the CT858 ORF.

SEQ ID NO: 89 is the determined amino acid sequence for the $\underline{\textit{C. pnuemoniae}}$ clone Cp_SWIB-His.

SEQ ID NO: 90 is the determined amino acid sequence for the <u>C. trachomatis</u>
<u>LGV II</u> clone CtL2_LPDA_FL.

SEQ ID NO: 91 is the determined amino acid sequence for the <u>C. pnuemoniae</u> clone CpS13-His.

SEQ ID NO: 92 is the determined amino acid sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone CtL2_TSA_FL.

SEQ ID NO: 93 is the amino acid sequence for Ct-Swib 43-61 peptide from C. trachomatis LGV II.

SEQ ID NO: 94 is the amino acid sequence for Ct-Swib 48-67 peptide from C. trachomatis LGV II.

SEQ ID NO: 95 is the amino acid sequence for Ct-Swib 52-71 peptide from *C. trachomatis* LGV II.

SEQ ID NO: 96 is the amino acid sequence for Ct-Swib 58-77 peptide from C. trachomatis LGV II.

SEQ ID NO: 97 is the amino acid sequence for Ct-Swib 63-82 peptide from C. trachomatis LGV II.

SEQ ID NO: 98 is the amino acid sequence for Ct-Swib 51-66 peptide from C. trachomatis LGV II.

SEQ ID NO: 99 is the amino acid sequence for Cp-Swib 52-67 peptide from C. pneumonia.

SEQ ID NO: 100 is the amino acid sequence for Cp-Swib 37-51 peptide from *C. pneumonia*.

SEQ ID NO: 101 is the amino acid sequence for Cp-Swib 32-51 peptide from *C. pneumonia*.

SEQ ID NO: 102 is the amino acid sequence for Cp-Swib 37-56 peptide from *C. pneumonia*.

SEQ ID NO: 103 is the amino acid sequence for Ct-Swib 36-50 peptide from C. trachomatis.

SEQ ID NO: 104 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis.

SEQ ID NO: 105 is the amino acid sequence for Ct-S13 60-80 peptide from C. trachomatis.

SEQ ID NO: 106 is the amino acid sequence for Ct-S13 1-20 peptide from C. trachomatis.

SEQ ID NO: 107 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis.

SEQ ID NO: 108 is the amino acid sequence for Ct-S13 56-75 peptide from C. trachomatis.

SEQ ID NO: 109 is the amino acid sequence for Cp-S13 56-75 peptide from *C. pneumoniae*.

SEQ ID NO: 110 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 21-G12-60, containing partial open reading frames for hypothetical proteins CT875, CT229 and CT228.

SEQ ID NO: 111 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 22-B3-53, sharing homology to the CT110 ORF of GroEL.

SEQ ID NO: 112 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 22-A1-49, sharing partial homology to the CT660 and CT659 ORFs.

SEQ ID NO: 113 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 17-E2-9, sharing partial homology to the CT611 and CT 610 ORFs.

SEQ ID NO: 114 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 17-C10-31, sharing partial homology to the CT858 ORF.

SEQ ID NO: 115 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 21-C7-66, sharing homology to the dnaK-like gene.

SEQ ID NO: 116 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 20-G3-45, containing part of the pmpB gene CT413. SEQ ID NO: 117 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 18-C5-2, sharing homology to the S1 ribosomal protein ORF.

SEQ ID NO: 118 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 17-C5-19, containing part of the ORFs for CT431 and CT430.

SEQ ID NO: 119 is the determined DNA sequence for the <u>C. trachomatis</u> <u>LGV II</u> clone 16-D4-22, contains partial sequences of ORF3 and ORF4 of the plasmid for growth within mammalian cells.

SEQ ID NO: 120 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar LGV II</u> Cap1 gene CT529.

SEQ ID NO: 121 is the predicted full-length amino acid sequence for the \underline{C} . $\underline{trachomatis}$ serovar LGV II Cap1 gene CT529.

SEQ ID NO: 122 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar E</u> Cap1 gene CT529.

SEQ ID NO: 123 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar E</u> Cap1 gene CT529.

SEQ ID NO: 124 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar 1A</u> Cap1 gene CT529.

SEQ ID NO: 125 is the predicted full-length amino acid sequence for the \underline{C} . $\underline{trachomatis}$ serovar 1A Cap1 gene CT529.

SEQ ID NO: 126 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar G Cap1 gene CT529.</u>

SEQ ID NO: 127 is the predicted full-length amino acid sequence for the \underline{C} . $\underline{trachomatis}$ serovar \underline{G} Cap1 gene CT529.

SEQ ID NO: 128 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis</u> serovar F1 NII Cap1 gene CT529.

SEQ ID NO: 129 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar F1 NII</u> Cap1 gene CT529.

SEQ ID NO: 130 is the determined full-length DNA sequence for the \underline{C} . $\underline{trachomatis}$ serovar L1 Cap1 gene CT529.

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SEQ ID NO: 131 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar L1 Cap1 gene CT529.</u>

SEQ ID NO: 132 is the determined full-length DNA sequence for the \underline{C} . $\underline{trachomatis}$ serovar L3 Cap1 gene CT529.

SEQ ID NO: 133 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar L3 Cap1</u> gene CT529.

SEQ ID NO: 134 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar Ba</u> Cap1 gene CT529.

SEQ ID NO: 135 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar Ba</u> Cap1 gene CT529.

SEQ ID NO: 136 is the determined full-length DNA sequence for the <u>C.</u> <u>trachomatis serovar MOPN</u> Cap1 gene CT529.

SEQ ID NO: 137 is the predicted full-length amino acid sequence for the <u>C.</u> <u>trachomatis serovar MOPN</u> Cap1 gene CT529.

SEQ ID NO: 138 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #124-139 of *C. trachomatis* serovar L2.

SEQ ID NO: 139 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #132-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 140 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-155 of *C. trachomatis* serovar L2.

SEQ ID NO: 141 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #146-163 of *C. trachomatis* serovar L2.

SEQ ID NO: 142 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #154-171 of *C. trachomatis* serovar L2.

SEQ ID NO: 143 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #162-178 of *C. trachomatis* serovar L2.

SEQ ID NO: 144 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 145 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #139-147 of *C. trachomatis* serovar L2.

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SEQ ID NO: 146 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #140-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 147 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-146 of *C. trachomatis* serovar L2.

SEQ ID NO: 148 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-145 of *C. trachomatis* serovar L2.

SEQ ID NO: 149 is the determined amino acid sequence for the Cap1 CT529 ORF peptide # F140->I of *C. trachomatis* serovar L2.

SEQ ID NO: 150 is the determined amino acid sequence for the Cap1 CT529 ORF peptide ##S139>Ga of *C. trachomatis* serovar L2.

SEQ ID NO: 151 is the determined amino acid sequence for the Cap1 CT529 ORF peptide ##S139>Gb of *C. trachomatis* serovar L2.

SEQ ID NO: 152 is the determined amino acid sequence for the peptide # 2 C7.8-6 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 153 is the determined amino acid sequence for the peptide # 2 C7.8-7 of the 216aa ORF of *C. trachomatis* servoar L2.

SEQ ID NO: 154 is the determined amino acid sequence for the peptide # 2 C7.8-8 of the 216aa ORF of C. trachomatis servar L2.

SEQ ID NO: 155 is the determined amino acid sequence for the peptide # 2 C7.8-9 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 156 is the determined amino acid sequence for the peptide # 2 C7.8-10 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 157 is the determined amino acid sequence for the 53 amino acid residue peptide of the 216aa ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 158 is the determined amino acid sequence for the 52 amino acid residue peptide of the CT529 ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 159 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 160 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 161 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 for serovars other than L2 and MOPN.

SEQ ID NO: 162 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovars other than L2 and MOPN.

SEQ ID NO: 163 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 164 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 165 is the determined DNA sequence for the 5' (forward) primer for pBIB-KS.

SEQ ID NO: 166 is the determined DNA sequence for the 5' (reverse) primer for pBIB-KS.

SEQ ID NO: 167 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar L2.

SEQ ID NO: 168 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar D.

SEQ ID NO: 169 is the determined full-length DNA sequence for the *C. trachomatis* pmpI gene.

SEQ ID NO: 170 is the determined full-length DNA sequence for the C. $trachomatis\ pmpG$ gene.

SEQ ID NO: 171 is the determined full-length DNA sequence for the $\it C$. $\it trachomatis$ pmpE gene.

SEQ ID NO: 172 is the determined full-length DNA sequence for the C. $trachomatis\ pmpD$ gene.

SEQ ID NO: 173 is the determined full-length DNA sequence for the $\it C$. $\it trachomatis pmpC$ gene.

SEQ ID NO: 174 is the determined full-length DNA sequence for the C. trachomatis pmpB gene.

SEQ ID NO: 175 is the predicted full-length amino acid sequence for the *C. trachomatis* pmpI gene.

SEQ ID NO: 176 is the predicted full-length amino acid sequence for the *C. trachomatis*-pmpG gene.

SEQ ID NO: 177 is the predicted full-length amino acid sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 178 is the predicted full-length amino acid sequence for the C. $trachomatis\ pmpD\ gene.$

SEQ ID NO: 179 is the predicted full-length amino acid sequence for the C. $trachomatis\ pmpC$ gene.

SEQ ID NO: 180 is the predicted full-length amino acid sequence for the C. $trachomatis\ pmpB$ gene.

SEQ ID NO: 181 is the determined DNA sequence minus the signal sequence for the *C. trachomatis* pmpI gene.

SEQ ID NO: 182 is a subsequently determined full-length DNA sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 183 is the determined DNA sequence minus the signal sequence for the *C. trachomatis* pmpE gene.

SEQ ID NO: 184 is a first determined DNA sequence representing the carboxy terminus for the *C. trachomatis* pmpD gene.

SEQ ID NO: 185 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the C. trachomatis pmpD gene.

SEQ ID NO: 186 is a first determined DNA sequence representing the carboxy terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 187 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the C. trachomatis pmpC gene.

SEQ ID NO: 188 is the determined DNA sequence representing the *C. pneumoniae* serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 189 is the predicted amino acid sequence minus the signal sequence for the *C. trachomatis* pmpI gene.

SEQ ID NO: 190 is subsequently predicted amino acid sequence for the C. $trachomatis\ pmpG$ gene.

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SEQ ID NO: 191 is the predicted amino acid sequence minus the signal sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 192 is a first predicted amino acid sequence representing the carboxy terminus for the *C. trachomatis* pmpD gene.

SEQ ID NO: 193 is a second predicted amino acid sequence representing the Amino terminus minus the signal sequence for the *C. trachomatis* pmpD gene.

SEQ ID NO: 194 is a first predicted amino acid sequence representing the Carboxy terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 195 is a second predicted amino acid sequence representing the Amino terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 196 is the predicted amino acid sequence representing the *C. pneumoniae* serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 197 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpC gene in the SKB vaccine vector.

SEQ ID NO: 198 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

SEQ ID NO: 199 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpC gene in the SKB vaccine vector.

SEQ ID NO: 200 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.

SEQ ID NO: 201 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.

SEQ ID NO: 202 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpD gene in the SKB vaccine vector.

SEQ ID NO: 203 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpE gene in the SKB vaccine vector.

SEQ ID NO: 204 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpE gene in the SKB vaccine vector.

SEQ ID NO: 205 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.

SEQ ID NO: 206 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpG gene in the SKB vaccine vector.

SEQ ID NO: 207 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 208 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 209 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 210 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 211 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 212 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 213 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 214 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 215 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

SEQ ID NO: 216 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 217 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

SEQ ID NO: 218 is the amino acid sequence for the insertion sequence for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 219 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

SEQ ID NO: 220 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

SEQ ID NO: 221 is the amino acid sequence for the insertion sequence for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

SEQ ID NO: 222 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpI gene in the pET17b vector.

SEQ ID NO: 223 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpI gene in the pET17b vector.

SEQ ID NO: 224 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 1-20.

SEQ ID NO: 225 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 6-25.

SEQ ID NO: 226 is the determined amino acid sequence for the C. pneumoniae Swib peptide 12-31.

SEQ ID NO: 227 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 17-36.

SEQ ID NO: 228 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 22-41.

SEQ ID NO: 229 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 27-46.

SEQ ID NO: 230 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 42-61.

SEQ ID NO: 231 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 46-65.

SEQ ID NO: 232 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 51-70.

SEQ ID NO: 233 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 56-75.

SEQ ID NO: 234 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 61-80.

SEQ ID NO: 235 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 66-87.

SEQ ID NO: 236 is the determined amino acid sequence for the *C. trachomatis*-OMCB-peptide 103-122.

SEQ ID NO: 237 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 108-127.

SEQ ID NO: 238 is the determined amino acid sequence for the C. trachomatis OMCB peptide 113-132.

SEQ ID NO: 239 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 118-137.

SEQ ID NO: 240 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 123-143.

SEQ ID NO: 241 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 128-147.

SEQ ID NO: 242 is the determined amino acid sequence for the C. trachomatis OMCB peptide 133-152.

SEQ ID NO: 243 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 137-156.

SEQ ID NO: 244 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 142-161.

SEQ ID NO: 245 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 147-166.

SEQ ID NO: 246 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 152-171.

SEQ ID NO: 247 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 157-176.

SEQ ID NO: 248 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 162-181.

SEQ ID NO: 249 is the determined amino acid sequence for the $\it C$. trachomatis OMCB peptide 167-186.

SEQ ID NO: 250 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 171-190.

SEQ ID NO: 251 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 171-186.

SEQ ID NO: 252 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 175-186.

SEQ ID NO: 252 is the determined amino acid sequence for the C. trachomatis OMCB peptide 175-186.

SEQ ID NO: 253 is the determined amino acid sequence for the *C. pneumoniae* OMCB peptide 185-198.

SEQ ID NO: 254 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 96-115.

SEQ ID NO: 255 is the determined amino acid sequence for the C. trachomatis TSA peptide 101-120.

SEQ ID NO: 256 is the determined amino acid sequence for the C. trachomatis TSA peptide 106-125.

SEQ ID NO: 257 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 111-130.

SEQ ID NO: 258 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 116-135.

SEQ ID NO: 259 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 121-140.

SEQ ID NO: 260 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 126-145.

SEQ ID NO: 261 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 131-150.

SEQ ID NO: 262 is the determined amino acid sequence for the C. trachomatis TSA peptide 136-155.

SEQ ID NO: 263 is the determined full-length DNA sequence for the C. trachomatis CT529/Cap 1 gene serovar I.

SEQ ID NO: 264 is the predicted full-length amino sequence for the *C. trachomatis* CT529/Cap 1 gene serovar I.

SEQ ID NO: 265 is the determined full-length DNA sequence for the C. -trachomatis CT529/Cap 1 gene serovar K.

SEQ ID NO: 266 is the predicted full-length amino sequence for the *C. trachomatis* CT529/Cap 1 gene serovar K.

SEQ ID NO: 267 is the determined DNA sequence for the *C. trachomatis* clone 17-G4-36 sharing homology to part of the ORF of DNA-dirrected RNA polymerase beta subunit- CT315 in serD.

SEQ ID NO: 268 is the determined DNA sequence for the partial sequence of the *C. trachomatis* CT016 gene in clone 2E10.

SEQ ID NO: 269 is the determined DNA sequence for the partial sequence of the *C. trachomatis* tRNA syntase gene in clone 2E10.

SEQ ID NO: 270 is the determined DNA sequence for the partial sequence for the *C. trachomatis* clpX gene in clone 2E10.

SEQ ID NO: 271 is a first determined DNA sequence for the *C. trachomatis* clone CtL2gam-30 representing the 5'end.

SEQ ID NO: 272 is a second determined DNA sequence for the *C. trachomatis* clone CtL2gam-30 representing the 3'end.

SEQ ID NO: 273 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-28.

SEQ ID NO: 274 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-27.

SEQ ID NO: 275 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-26.

SEQ ID NO: 276 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-24.

SEQ ID NO: 277 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-23.

SEQ ID NO: 278 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-21.

SEQ ID NO: 279 is the determined DNA sequence for the C. trachomatis

clone CtL2gam-18.

SEQ ID NO: 280 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-17.

SEQ ID NO: 281 is a first determined DNA sequence for the *C. trachomatis* clone CtL2gam-15 representing the 5' end.

SEQ ID NO: 282 is a second determined DNA sequence for the *C. trachomatis* clone CtL2gam-15 representing the 3' end.

SEQ ID NO: 283 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-13.

SEQ ID NO: 284 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-10.

SEQ ID NO: 285 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-8.

SEQ ID NO: 286 is a first determined DNA sequence for the *C. trachomatis* clone CtL2gam-6 representing the 5' end.

SEQ ID NO: 287 is a second determined DNA sequence for the *C. trachomatis* clone CtL2gam-6 representing the 3' end.

SEQ ID NO: 288 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-5.

SEQ ID NO: 289 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-2.

SEQ ID NO: 290 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-1.

SEQ ID NO: 291 is the determined full-length DNA sequence for the *C. pneumoniae* homologue of the CT529 gene.

SEQ ID NO: 292 is the predicted full-length amino acid sequence for the *C. pneumoniae* homologue of the CT529 gene.

SEQ ID NO: 293 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpG gene in the SKB vaccine vector.

DESCRIPTION OF THE FIGURES

Fig. 1 illustrates induction of INF- γ from a *Chlamydia*-specific T cell line activated by target cells expressing clone 4C9-18#2.

- Fig. 2 illustrates retroviral vectors pBIB-KS1,2,3 modified to contain a Kosak translation initiation site and stop codons.
- Fig. 3 shows specific lysis in a chromium release assay of P815 cells pulsed with *Chlamydia* peptides CtC7.8-12 (SEQ ID NO: 18) and CtC7.8-13 (SEQ ID NO: 19).
- Fig. 4 shows antibody isotype titers in C57Bl/6 mice immunized with *C. trachomatis* SWIB protein.
- Fig. 5 shows *Chlamydia*-specific T-cell proliferative responses in splenocytes from C3H mice immunized with *C. trachomatis* SWIB protein.
- Fig. 6 illustrates the 5' and 3' primer sequences designed from *C. pneumoniae* which were used to isolate the SWIB and S13 genes from *C. pneumoniae*.
- Figs. 7A and 7B show induction of IFN-γ from a human anti-chlamydia T-cell line (TCL-8) capable of cross-reacting to *C. trachomatis* and *C. pneumonia* upon activation by monocyte-derived dendritic cells expressing chlamydial proteins.
- Fig. 8 shows the identification of T cell epitopes in Chlamydial ribosomal S13 protein with T-cell line TCL 8 EB/DC.
- Fig. 9 illustrates the proliferative response of CP-21 T-cells generated against *C. pnuemoniae*-infected dendritic cells to recombinant *C. pneumonia*-SWIBprotein, but not *C. trachomatis* SWIB protein.
- Fig. 10 shows the *C. trachomatis*-specific SWIB proliferative responses of a primary T-cell line (TCT-10 EB) from an asymptomatic donor.
- Fig. 11 illustrates the identification of T-cell epitope in *C. trachomatis* SWIB with an antigen specific T-cell line (TCL-10 EB).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the diagnosis and treatment of Chlamydial infection. In one aspect, the compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a *Chlamydia* antigen, or a variant thereof.

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In specific embodiments, the subject invention discloses polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, wherein the *Chlamydia* antigen comprises an amino acid sequence encoded by a polynucleotide molecule including a sequence selected from the group consisting of (a) nucleotide sequences recited in SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290 (b) the complements of said nucleotide sequences, and (c) variants of such sequences.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e., antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the inventive antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *Chlamydia* antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

An "immunogenic portion" of an antigen is a portion that is capable of reacting with sera obtained from a *Chlamydia*-infected individual (*i.e.*, generates an absorbance reading with sera from infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). Such immunogenic portions generally comprise at least about 5 amino acid residues, more preferably at least about 10, and most

preferably at least about 20 amino acid residues. Methods for preparing and identifying immunogenic portions of antigens of known sequence are well known in the art and include those summarized in Paul, Fundamental Immunology, 3rd ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native Chlamydia protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ¹²⁵I-labeled Protein A.

Examples of immunogenic portions of antigens contemplated by the present invention include, for example, the T cell stimulating epitopes provided in SEQ ID NO: 9, 10, 18, 19, 31, 39, 93-96, 98, 100-102, 106, 108, 138-140, 158, 167, 168, 246, 247 and 254-256. Polypeptides comprising at least an immunogenic portion of one or more *Chlamydia* antigens as described herein may generally be used, alone or in combination, to detect Chlamydial infection in a patient.

The compositions and methods of the present invention also encompass variants of the above polypeptides and polynucleotide molecules. Such variants include, but are not limited to, naturally occurring allelic variants of the inventive sequences. In particular, variants include other *Chlamydiae* serovars, such as serovars D, E and F, as well as the several LGV serovars which share homology to the inventive polypeptide and

polynucleotide molecules described herein. Preferably, the serovar homologues show 95-99% homology to the corresponding polypeptide sequence(s) described herein.

A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such that the antigenic properties of the polypeptide are retained. In a preferred embodiment, variant polypeptides differ from an identified sequence by substitution, deletion or addition of five amino acids or fewer. Such variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the antigenic properties of the modified polypeptide using, for example, the representative procedures described herein. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (e.g., 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein. Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity (determined as described below) to the identified polypeptides.

As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent

conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val. ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or In a preferred embodiment, variant alternatively, contain nonconservative changes. polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide. Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A polynucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions such that the immunogenicity of the encoded polypeptide is not diminished, relative to the native protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis as taught, for example, by Adelman et al. (*DNA*, 2:183, 1983). Nucleotide variants may be naturally occurring allelic variants as discussed below, or non-naturally occurring variants. Variant nucleotide sequences preferably exhibit at least about 70%, more preferably at least about 80% and most preferably at least about 90% identity (determined as described below) to the recited sequence.

The polypeptides provided by the present invention include variants that are encoded by polynucleotide sequences which are substantially homologous to one or more of the polynucleotide sequences specifically recited herein. "Substantial homology," as used herein, refers to polynucleotide sequences that are capable of hybridizing under moderately

stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species homology, at 45°C with 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Such hybridizing polynucleotide sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode a polypeptide that is the same as a polypeptide of the present invention.

Two nucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Resarch Foundaiton, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) Fast and sensitive multiple sequence alignments on a microcomputer CABIOS 5:151-153; Myers, E.W. and Muller W. (1988) Optimal alignments in linear space CABIOS 4:11-17; Robinson, E.D. (1971) Comb. Theor 11:105; Santou, N. Nes, M. (1987) The neighbor joining method. A new method for reconstructing phylogenetic trees Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy, Freeman Press, San

Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) Rapid similarity searches of nucleic acid and protein data banks *Proc. Natl. Acad., Sci. USA* 80:726-730.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Also included in the scope of the present invention are alleles of the genes encoding the nucleotide sequences recited in herein. As used herein, an "allele" or "allellic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone or in combination with the others, one or more times in a given sequence. In specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a Chlamydia antigen (or a variant of such an antigen), that comprises one or more of the amino acid sequences encoded by (a) a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1-4, 15 21-25, 44-64, 66-76 and 79-88; (b) the complements of such DNA sequences or (c) DNA sequences substantially homologous to a sequence in (a) or (b). As discussed in the Examples below, several of the Chlamydia antigens disclosed herein recognize a T cell line that recognizes both Chlamydia trachomatis and Chlamydia pneumoniae infected monocyte-derived dendritic cells, indicating that they may represent an immunoreactive epitope shared by Chlamydia trachomatis and WO 00/34483 PCT/US99/29012

Chlamydia pneumoniae. The antigens may thus be employed in a vaccine for both C. trachomatis genital tract infections and for C. pneumonia infections. Further characterization of these Chlamydia antigens from Chlamydia trachomatis and Chlamydia pneumonia to determine the extent of cross-reactivity is provided in Example 6. Additionally, Example 4 describes cDNA fragments (SEQ ID NO: 15, 16 and 33) isolated from C. trachomatis which encode proteins (SEQ ID NO: 17-19 and 32) capable of stimulating a Chlamydia-specific murine CD8+T cell line.

In general, Chlamydia antigens, and polynucleotide sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, polynucleotide molecules encoding Chlamydia antigens may be isolated from a Chlamydia genomic or cDNA expression library by screening with a Chlamydia-specific T cell line as described below, and sequenced using techniques well known to those of skill in the art. Additionally, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for Chlamydia-associated expression (i.e., expression that is at least two fold greater in Chlamydia-infected cells than in controls, as determined using a representative assay provided herein). Such screens may be performed using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., Proc. Natl. Acad. Sci. USA 93:10614-10619, 1996 and Heller et al., Proc. Natl. Acad. Sci. USA 94:2150-2155, 1997). Alternatively, polypeptides may be amplified from cDNA prepared from cells expressing the proteins described herein. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

Antigens may be produced recombinantly, as described below, by inserting a polynucleotide sequence that encodes the antigen into an expression vector and expressing the antigen in an appropriate host. Antigens may be evaluated for a desired property, such as the ability to react with sera obtained from a *Chlamydia*-infected individual as described herein, and may be sequenced using, for example, traditional Edman chemistry. *See* Edman and Berg, *Eur. J. Biochem.* 80:116-132, 1967.

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Polynucleotide sequences encoding antigens may also be obtained by screening an appropriate *Chlamydia* cDNA-or genomic DNA library for polynucleotide sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated antigens. Degenerate oligonucleotide sequences for use in such a screen may be designed and synthesized, and the screen may be performed, as described (for example) in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using the above oligonucleotides in methods well known in the art, to isolate a nucleic acid probe from a cDNA or genomic library. The library screen may then be performed using the isolated probe.

An amplified portion may be used to isolate a full length gene from a suitable library (e.g., a Chlamydia cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ³²P) using well known techniques. A bacterial or bacteriophage library is then screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences are then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known

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techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using techniques well known in the art (see, for example, Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263, 1987; Erlich ed., PCR Technology, Stockton Press, NY, 1989), and software well known in the art may also be employed. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (see Triglia et al., Nucl. Acids Res. 16:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Additional techniques include capture PCR (Lagerstrom et al., PCR Methods Applic. 1:111-19, 1991) and walking PCR (Parker et al., Nucl. Acids. Res. 19:3055-60, 1991). Transcription-Mediated Amplification, or TMA is another method that may be utilized for the amplification of DNA, rRNA, or mRNA, as described in Patent No. PCT/US91/03184. This autocatalytic and isothermic non-PCR based method utilizes two primers and two enzymes: RNA polymerase and reverse transcriptase. One primer contains a promoter sequence for RNA polymerase. In the first amplification, the promoter-primer hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the 3'end of the promoter-primer. The RNA in the resulting complex is degraded and a second primer binds to the DNA copy. A

new strand of DNA is synthesized from the end of the primer by reverse transcriptase creating double stranded DNA. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA amplicons re-enters the TMA process and serves as a template for a new round of replication leading to the expotential expansion of the RNA amplicon. Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length cDNA sequences may also be obtained by analysis of genomic fragments.

Polynucleotide variants may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (see Adelman et al., DNA 2:183, 1983). Alternatively, RNA molecules may be generated by in vitro or in vivo transcription of DNA sequences encoding a Chlamydial protein, or portion thereof, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated in vivo (e.g., by transfecting antigen-presenting cells, such as dendritic cells, with a cDNA construct encoding a Chlamydial polypeptide, and administering the transfected cells to the patient).

A portion of a sequence complementary to a coding sequence (i.e., an antisense polynucleotide) may also be used as a probe or to modulate gene expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells of tissues to facilitate the production of antisense RNA. An antisense polynucleotide may be used, as described herein, to inhibit expression of a *Chlamydial* protein. Antisense technology can be used to control gene expression through triple-helix formation, which

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compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (see Gee et al., In Huber and Carr, Molecular and Immunologic Approaches, Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (e.g., promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

A portion of a coding sequence, or of a complementary sequence, may also be designed as a probe or primer to detect gene expression. Probes may be labeled with a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers, as noted above, are preferably 22-30 nucleotides in length.

Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where

amino acids are sequentially added to a growing amino acid chain. See Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division, Foster City, CA, and may be operated according to the manufacturer's instructions.

As noted above, immunogenic portions of *Chlamydia* antigens may be prepared and identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3d ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptide portions of the native antigen for immunogenic properties. The representative ELISAs described herein may generally be employed in these screens. An immunogenic portion of a polypeptide is a portion that, within such representative assays, generates a signal in such assays that is substantially similar to that generated by the full length antigen. In other words, an immunogenic portion of a *Chlamydia* antigen generates at least about 20%, and preferably about 100%, of the signal induced by the full length antigen in a model ELISA as described herein.

Portions and other variants of *Chlamydia* antigens may be generated by synthetic or recombinant means. Variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Sections of the polynucleotide sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

Recombinant polypeptides containing portions and/or variants of a native antigen may be readily prepared from a polynucleotide sequence encoding the polypeptide using a variety of techniques well known to those of ordinary skill in the art. For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides as described herein. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an

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expression vector containing a polynucleotide molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line, such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in an isolated, substantially pure, form. Preferably, the polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known Chlamydial protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein. A DNA sequence encoding a fusion protein of the present invention may be constructed using known recombinant DNA techniques to assemble separate DNA sequences encoding, for example, the first and second polypeptides, into an appropriate expression vector. The 3' end of a DNA sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the

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fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., Gene 40:39-46, 1985; Murphy et al., Proc. Natl. Acad. Sci. USA 83:8258-8562, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. As an alternative to the use of a peptide linker sequence (when desired), one can utilize non-essential N-terminal amino acid regions (when present) on the first and second polypeptides to separate the functional domains and prevent steric hindrance.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided that comprise a polypeptide of the present invention together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (see, for example, Stoute et al. New Engl. J. Med., 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (e.g., the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in E. coli (thus functioning as

an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene 43*:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology 10*:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305. Additionally, the fusion protein Ra12 may be linked to the inventive polynucleotides to facilitate protein expression.

In another aspect, the present invention provides methods for using one or more of the above polypeptides or fusion proteins (or polynucleotides encoding such polypeptides or fusion proteins) to induce protective immunity against Chlamydial infection in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat Chlamydial infection.

In this aspect, the polypeptide, fusion protein or polynucleotide molecule is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines may comprise one or more of the above polypeptides and an immunostimulant,

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such as an adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other *Chlamydia* antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

Alternatively, a vaccine may contain polynucleotides encoding one or more polypeptides or fusion proteins as described above, such that the polypeptide is generated in situ. In such vaccines, the polynucleotides may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary polynucleotide sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the polynucleotides may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective) virus. Techniques for incorporating polynucleotides into such expression systems are well known to those of ordinary skill in the art. The polynucleotides may also be administered as "naked" plasmid vectors as described, for example, in Ulmer et al., Science 259:1745-1749, 1993 and reviewed by Cohen, Science 259:1691-1692, 1993. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The uptake of naked polynucleotides may be increased by incorporating the polynucleotides into and/or onto biodegradable beads, which are efficiently transported into the cells. The preparation and use of such systems is well known in the art.

In a related aspect, a polynucleotide vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known *Chlamydia* antigen. For example, administration of polynucleotides encoding a polypeptide of the present invention, either "naked" or in a delivery system as described above, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Polypeptides and polynucleotides disclosed herein may also be employed in adoptive immunotherapy for the treatment of *Chlamydial* infection. Adoptive immunotherapy may be broadly classified into either active or passive immunotherapy. In active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system with the administration of immune response-modifying agents (for example, vaccines, bacterial adjuvants, and/or cytokines).

In passive immunotherapy, treatment involves the delivery of biologic reagents with established immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate anti-*Chlamydia* effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocyte, CD4+ T-helper), killer cells (such as Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-idiotypic antibodies (as in U.S. Patent No. 4,918,164), for passive immunotherapy.

The predominant method of procuring adequate numbers of T-cells for adoptive immunotherapy is to grow immune T-cells *in vitro*. Culture conditions for expanding single antigen-specific T-cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. These *in vitro* culture conditions typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast, or B-cells, may be pulsed with

immunoreactive polypeptides, or polynucleotide sequence(s) may be introduced into antigen presenting cells, using a variety of standard techniques well known in the art. For example, antigen presenting cells may be transfected or transduced with a polynucleotide sequence, wherein said sequence contains a promoter region appropriate for increasing expression, and can be expressed as part of a recombinant virus or other expression system. Several viral vectors may be used to transduce an antigen presenting cell, including pox virus, vaccinia virus, and adenovirus; also, antigen presenting cells may be transfected with polynucleotide sequences disclosed herein by a variety of means, including gene-gun technology, lipidmediated delivery, electroporation, osmotic shock, and particlate delivery mechanisms, resulting in efficient and acceptable expression levels as determined by one of ordinary skill in the art. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term in vivo. Studies have demonstrated that cultured T-cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever, M., et al, "Therapy With Cultured T Cells: Principles Revisited," Immunological Reviews, 157:177, 1997).

The polypeptides disclosed herein may also be employed to generate and/or isolate chlamydial-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by *in vivo* immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ or CD4+ T-cell clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

Alternatively, peptides corresponding to immunogenic portions of the polypeptides may be employed to generate *Chlamydia* reactive T cell subsets by selective *in vitro* stimulation and expansion of autologous T cells to provide antigen-specific T cells which may be subsequently transferred to the patient as described, for example, by Chang *et al, (Crit. Rev. Oncol. Hematol., 22*(3), 213, 1996). Cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as IsolexTM System, available from Nexell Therapeutics, Inc. Irvine, CA. The separated cells are stimulated with one or more of the immunoreactive

polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T-cells. The population of antigen-specific T cells is then expanded using standard techniques and the cells are administered back to the patient.

In other embodiments, T-cell and/or antibody receptors specific for the polypeptides disclosed herein can be cloned, expanded, and transferred into other vectors or effector cells for use in adoptive immunotherapy. In particular, T cells may be transfected with the appropriate genes to express the variable domains from chlamydia specific monoclonal antibodies as the extracellular recognition elements and joined to the T cell receptor signaling chains, resulting in T cell activation, specific lysis, and cytokine release. This enables the T cell to redirect its specificity in an MHC-independent manner. See for example, Eshhar, Z., Cancer Immunol Immunother, 45(3-4):131-6, 1997 and Hwu, P., et al, Cancer Res, 55(15):3369-73, 1995. Another embodiment may include the transfection of chlamydia antigen specific alpha and beta T cell receptor chains into alternate T cells, as in Cole, DJ, et al, Cancer Res, 55(4):748-52, 1995.

In a further embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides corresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-specific dendritic cells may either be transferred into a patient, or employed to stimulate T cells to provide antigen-specific T cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T cells and the subsequent use of such antigen-specific T cells to eradicate disease in a murine model has been demonstrated by Cheever et al, *Immunological Reviews*, 157:177, 1997). Additionally, vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated *in vitro* for autologous transplant back into the same patient.

Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents disclosed herein may be incorporated into pharmaceutical compositions or immunogenic compositions (*i.e.*, vaccines). Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds and an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response to an exogenous antigen.

Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g., polylactic galactide) and liposomes (into which the compound is incorporated; see e.g., Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other *Chlamydial* antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated in situ. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, Crit. Rev. Therap. Drug Carrier Systems 15:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch et al., Proc. Natl. Acad. Sci. USA 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, Biotechniques 6:616-627, 1988; Rosenfeld et al., Science 252:431-434, 1991; Kolls et al., Proc. Natl. Acad. Sci. USA 91:215-219, 1994; Kass-Eisler et al., Proc. Natl. Acad. Sci. USA 90:11498-11502, 1993; Guzman et al., Circulation 88:2838-2848, 1993; and Guzman et al., Cir. Res. 73:1202-1207, 1993.

Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill-in-the-art. The DNA may also-be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide) and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially available

as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, under select circumstances, the adjuvant composition may be designed to induce an immune response predominantly of the Th1 type or Th2 type. High levels of Th1-type cytokines (e.g., IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Ribi ImmunoChem Research Inc. (Hamilton, MT) (see US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555. Another preferred adjuvant is a saponin, preferably QS21, which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations

comprises an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210. Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient.

The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets *Chlamydia*-infected cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-*Chlamydia* effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs

(Banchereau and Steinman, Nature 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic immunity (see Timmerman and Levy, Ann. Rev. Med. 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate in situ, with marked cytoplasmic processes (dendrites) visible in vitro), their ability to take up, process and present antigens with high efficiency, and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells in vivo or ex vivo, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (see Zitvogel et al., Nature Med. 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcy receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a

Chlamydial protein (or portion or other variant thereof) such that the Chlamydial polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place ex vivo, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs in vivo. In vivo and ex vivo transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., Immunology and cell Biology 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the Chlamydial polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a nonconjugated immunological partner, separately or in the presence of the polypeptide.

Routes and frequency of administration of pharmaceutical compositions and vaccines, as well as dosage, will vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (e.g., intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from *Chlamydial* infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 µg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

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While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a *Chlamydial* protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

In another aspect, the present invention provides methods for using the polypeptides described above to diagnose Chlamydial infection. In this aspect, methods are provided for detecting Chlamydial infection in a biological sample, using one or more of the above polypeptides, either alone or in combination. For clarity, the term "polypeptide" will be used when describing specific embodiments of the inventive diagnostic methods. However, it will be clear to one of skill in the art that the fusion proteins of the present invention may also be employed in such methods.

As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient. The polypeptides are used in an assay, as described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative

to a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to *Chlamydia* antigens which may be indicative of *Chlamydia*-infection.

In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (i.e., one component polypeptide will tend to detect infection in samples where the infection would not be detected by another component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with *Chlamydia*. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested.

A variety of assay formats are known to those of ordinary skill in the art for using one or more polypeptides to detect antibodies in a sample. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988, which is incorporated herein by reference. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (e.g., in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate, or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The polypeptides may be bound to the solid support using a variety of techniques known to those of ordinary skill in the art. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 µg, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin (BSA) or Tween 20TM (Sigma Chemical Co., St. Louis, MO) may be employed. The

immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable dilutent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (i.e., incubation time) is that period of time that is sufficient to detect the presence of antibody within an HGE-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (e.g., Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods

MANGARAN PENGKANAN BERBARAN BERBARAN BERBARAN BERBARAN BERBARAN BERBARAN PENGKAN BERBARAN BERBAR BERBARAN BERBAR are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-Chlamydia antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for Chlamydia-infection. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., Clinical Epidemiology: A Basic Science for Clinical Medicine, Little Brown and Co., 1985, pp. 106-107. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (i.e., the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for Chlamydial infection.

In a related embodiment, the assay is performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (e.g., protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be

performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-*Chlamydia* antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 µg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (e.g., one drop) of patient serum or blood.

Of course, numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only. One example of an alternative assay protocol which may be usefully employed in such methods is a Western blot, wherein the proteins present in a biological sample are separated on a gel, prior to exposure to a binding agent. Such techniques are well known to those of skill in the art.

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a *Chlamydial* protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a *Chlamydial* protein if it reacts at a detectable level (within, for example, an ELISA) with a *Chlamydial* protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10³

L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a *Chlamydial* infection using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a *Chlamydial* protein will generate a signal indicating the presence of a *Chlamydial* infection in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without infection. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.*, blood, sera, sputum urine and/or tissue biopsies) from patients with and without *Chlamydial* infection (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and

the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A*

Laboratory Manual, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ⁹⁰Y, ¹²³I, ¹²⁵I, ¹³¹I, ¹⁸⁶Re, ¹⁸⁸Re, ²¹¹At, and ²¹²Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, e.g., U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion

of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (e.g., U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (e.g., U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (e.g., U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (e.g., U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (e.g., U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (e.g., U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (e.g., U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (e.g., U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent Nos. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent Nos. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or

in site-specific regions by appropriate methods. It will be evident that the precise dose of the antibody/immunoconjugate will-vary depending upon the antibody used, the antigen density, and the rate of clearance of the antibody.

Antibodies may be used in diagnostic tests to detect the presence of *Chlamydia* antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting Chlamydial infection in a patient.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify *Chlamydia*-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

As used herein, the term "oligonucleotide primer/probe specific for a DNA molecule" means an oligonucleotide sequence that has at least about 80%, preferably at least about 90% and more preferably at least about 95%, identity to the DNA molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (see, for example, Mullis *et al. Ibid*; Ehrlich, *Ibid*). Primers or probes may thus be used to detect *Chlamydia*-specific sequences in biological samples. DNA probes or primers

comprising oligonucleotide sequences described above may be used alone or in combination with each other.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

ISOLATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS

Chlamydia antigens of the present invention were isolated by expression cloning of a genomic DNA library of Chlamydia trachomatis LGV II essentially as described by Sanderson et al. (J. Exp. Med., 1995, 182:1751-1757) and were shown to induce PBMC proliferation and IFN-γ in an immunoreactive T cell line.

A Chlamydia-specific T cell line was generated by stimulating PBMCs from a normal donor with no history of chlamydial genital tract infection with elementary bodies of Chlamydia trachomatis LGV II. This T cell line, referred to as TCL-8, was found to recognize both Chlamydia trachomatis and Chlamydia pneumonia infected monocyte-derived dendritic cells.

A randomly sheared genomic library of *Chlamydia trachomatis* LGV II was constructed in Lambda ZAP (Stratagene, La Jolla, CA) and the amplified library plated out in 96 well microtiter plates at a density of 30 clones/well. Bacteria were induced to express recombinant protein in the presence of 2 mM IPTG for 3 h, then pelleted and resuspended in 200 μl of RPMI 10% FBS. 10 μl of the induced bacterial suspension was transferred to 96 well plates containing autologous monocyte-derived dendritic cells. After a 2 h incubation, dendritic cells were washed to remove free *E. coli* and *Chlamydia*-specific T cells were added. Positive *E. coli* pools were identified by determining IFN-γ production and proliferation of the T cells in response to the pools.

Four positive pools were identified, which were broken down to yield four pure clones (referred to as 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31), with insert sizes of 481 bp, 183 bp, 110 bp and 1400 bp, respectively. The determined DNA sequences for 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31 are provided in SEQ ID NO: 1-4, respectively. Clone 1-B1-66 is approximately in region 536690 of the *C. trachomatis* genome (NCBI *C.*

trachomatis database). Within clone 1-B1-66, an open reading frame (ORF) has been identified (nucleotides 115 - 375) that encodes a previously identified 9 kDa protein (Stephens, et al. Genbank Accession No. AE001320), the sequence of which is provided in SEQ ID NO: 5). Clone 4-D7-28 is a smaller region of the same ORF (amino acids 22-82 of 1-B1-66). Clone 3-G3-10 is approximately in region 74559 of the *C. trachomatis* genome. The insert is cloned in the antisense orientation with respect to its orientation in the genome. The clone 10-C10-31 contains an open reading frame that corresponds to a previously published sequence for S13 ribosomal protein from *Chlamydia trachomatis* (Gu, L. et al. *J. Bacteriology*, 177:2594-2601, 1995). The predicted protein sequences for 4-D7-28 and 10-C10-31 are provided in SEQ ID NO: 6 and 12, respectively. Predicted protein sequences for 3-G3-10 are provided in SEQ ID NO: 7-11.

In a related series of screening studies, an additional T cell line was used to screen the genomic DNA library of *Chlamydia trachomatis* LGV II described above. A *Chlamydia*-specific T cell line (TCT-1) was derived from a patient with a chlamydial genital tract infection by stimulating patient PBMC with autologous monocyte-derived dendritic cells infected with elementary bodies of *Chlamydia trachomatis* LGV II. One clone, 4C9-18 (SEQ ID NO: 21), containing a 1256 bp insert, elicited a specific immune response, as measured by standard proliferation assays, from the *Chlamydia*-specific T cell line TCT-1. Subsequent analysis revealed this clone to contain three known sequences: lipoamide dehydrogenase (Genbank Accession No. AE001326), disclosed in SEQ ID NO: 22; a hypothetical protein CT429 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 23; and part of an open reading frame of ubiquinone methyltransferase CT428 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 24.

In further studies involving clone 4C9-18 (SEQ ID NO: 21), the full-length amino acid sequence for lipoamide dehydrognase (SEQ ID NO: 22) from *C. trachomatis* (LGV II) was expressed in clone CtL2-LPDA-FL, as disclosed in SEQ ID NO: 90.

To further characterize the open reading frame containing the T cell stimulating epitope(s), a cDNA fragment containing nucleotides 1-695 of clone 4C9-18 with a cDNA sequence encoding a 6X-Histidine tag on the amino terminus was subcloned into the NdeI/EcoRI site of the pET17b vector (Novagen, Madison, WI), referred to as clone 4C9-

18#2 BL21 pLysS (SEQ ID NO: 25, with the corresponding amino acid sequence provided in SEQ ID NO: 26) and transformed into *E. coli*. Selective induction of the transformed *E. coli* with 2 mM IPTG for three hours resulted in the expression of a 26 kDa protein from clone 4C9-18#2 BL21 pLysS, as evidenced by standard Coomassie-stained SDS-PAGE. To determine the immunogenicity of the protein encoded by clone 4C9-18#2 BL21 pLysS, *E. coli* expressing the 26 kDa protein were titered onto 1 x 10⁴ monocyte-derived dendritic cells and incubated for two hours. The dendritic cell cultures were washed and 2.5 x 10⁴ T cells (TCT-1) added and allowed to incubate for an additional 72 hours, at which time the level of IFN-γ in the culture supernatant was determined by ELISA. As shown in Fig. 1, the T-cell line TCT-1 was found to respond to induced cultures as measured by IFN-g, indicating a *Chlamydia*-specific T-cell response against the lipoamide dehydrogenase sequence. Similarly, the protein encoded by clone 4C9-18#2 BL21 pLysS was shown to stimulate the TCT-1 T-cell line by standard proliferation assays.

Subsequent studies to identify additional *Chlamydia trachomatis* antigens using the above-described CD4+ T-cell expression cloning technique yielded additional clones. The TCT-1 and TCL-8 *Chlamydia*-specific T-cell lines, as well as the TCP-21 T-cell line were utilized to screen the *Chlamydia trachomatis* LGVII genomic library. The TCP-21 T-cell line was derived from a patient having a humoral immune response to *Chlamydia pnuemoniae*. The TCT-1 cell line identified 37 positive pools, the TCT-3 cell line identified 41 positive pools and the TCP-21 cell line identified 2 positive pools. The following clones were derived from 10 of these positive pools. Clone 11-A3-93 (SEQ ID NO: 64), identified by the TCP-21 cell line, is a 1339 bp genomic fragment sharing homology to the HAD superfamily (CT103). The second insert in the same clone shares homology with the fab I gene (CT104) present on the complementary strand. Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of *C. pnuemoniae*.

Clone 11-G10-46, (SEQ ID NO: 62), identified using the TCT-3 cell line, contains a 688 bp insert that shares homology to the hypothetical protein CT610. Clone 11-G1-34, (SEQ ID NO: 61), identified using the TCT-3 cell line, has two partial open reading

frames (ORF) with an insert size of 1215 bp. One ORF shares homology to the malate dehydrogenase gene (CT376), and the other ORF shares homology to the glycogen hydrolase gene (CT042). Clone 11-H3-68, (SEQ ID NO: 60), identified using the TCT-3 cell line, has two ORFs with a total insert size of 1180 bp. One partial ORF encodes the plasmid-encoded PGP6-D virulence protein while the second ORF is a complete ORF for the L1 ribosomal gene (CT318). Clone 11-H4-28, (SEQ ID NO: 59), identified using the TCT-3 cell line, has an insert size of 552 bp and is part of the ORF for the dnaK gene (CT396). Clone 12-B3-95, (SEQ ID NO: 58), identified using the TCT-1 cell line, has an insert size of 463 bp and is a part of the ORF for for the lipoamide dehydrogenase gene (CT557). Clones 15-G1-89 and 12-B3-95 are identical, (SEQ ID NO: 55 and 58, respectively), identified using the TCT-1 cell line, has an insert size of 463 bp and is part of the ORF for the lipoamide dehydrogenase gene (CT557). Clone 12-G3-83, (SEQ ID NO: 57), identified using the TCT-1 cell line, has an insert size of 1537 bp and has part of the ORF for the hypothetical protein CT622.

Clone 23-G7-68, (SEQ ID NO: 79), identified using the TCT-3 cell line, contains a 950 bp insert and contains a small part of the L11 ribosomal ORF, the entire ORF for L1 ribosomal protein and a part of the ORF for L10 ribosomal protein. Clone 22-F8-91, (SEQ ID NO: 80), identified using the TCT-1 cell line, contains a 395 bp insert that contains a part of the pmpC ORF on the complementary strand of the clone. Clone 21-E8-95, (SEQ ID NO: 81), identified using the TCT-3 cell line, contains a 2,085 bp insert which contains part of CT613 ORF, the complete ORF for CT612, the complete ORF for CT611 and part of the ORF for CT610. Clone 19-F12-57, (SEQ ID NO: 82), identified using the TCT-3 cell line, contains a 405 bp insert which contains part of the CT 858 ORF and a small part of the recA ORF. Clone 19-F12-53, (SEQ ID NO: 83), identified using the TCT-3 cell line, contains a 379 bp insert that is part of the ORF for CT455 encoding glutamyl tRNA synthetase. Clone 19-A5-54, (SEQ ID NO: 84), identified using the TCT-3 cell line, contains a 715 bp insert that is part of the ORF3 (complementary strand of the clone) of the cryptic plasmid. Clone 17-E11-72, (SEQ ID NO: 85), identified using the TCT-1 cell line, contains a 476 bp insert that is part of the ORF for Opp 2 and pmpD. The pmpD region of this clone is covered by the pmpD region of clone 15-H2-76. Clone 17-C1-77, (SEQ ID NO: 86), identified using the TCT-3 cell line, contains a 1551 bp insert that is part of the CT857 ORF, as well as part of the CT858 ORF. Clone 15-H2-76, (SEQ ID NO: 87), identified using the TCT-1 cell line, contains a 3,031 bp insert that contains a large part of the pmpD ORF, part of the CT089 ORF, as well as part of the ORF for SycE. Clone 15-A3-26, (SEQ ID NO: 88), contains a 976 bp insert that contains part of the ORF for CT858. Clone 17-G4-36, (SEQ ID NO: 267), identified using the TCT-10 cell line, contains a 680 bp insert that is in frame with beta-gal in the plasmid and shares homology to part of the ORF for DNA-directed RNA polymerase beta subunit (CT315 in SerD).

Several of the clones described above share homology to various polymorphic membrane proteins. The genomic sequence of *Chlamydia trachomatis* contains a family of nine polymorphic membrane protein genes, referred to as pmp. These genes are designated pmpA, pmpB, pmpC, pmpD, pmpE, pmpF, pmpG, pmpH and pmpI. Proteins expressed from these genes are believed to be of biological relevance in generating a protective immune response to a *Chlamydial* infection. In particular, pmpC, pmpD, pmpE and pmpI contain predictable signal peptides, suggesting they are outer membrane proteins, and therefore, potential immunological targets.

Based on the *Chlamydia trachomatis* LGVII serovar sequence, primer pairs were designed to PCR amplify the full-length fragments of pmpC, pmpD, pmpE, pmpG, pmpH and pmpI. The resulting fragments were subcloned into the DNA vaccine vector JA4304 or JAL, which is JA4304 with a modified linker (SmithKline Beecham, London, England). Specifically, PmpC was subcloned into the JAL vector using the 5' oligo GAT AGG CGC GCC GCA ATC ATG AAA TTT ATG TCA GCT ACT GCT G and the 3' oligo CAG AAC GCG TTT AGA ATG TCA TAC GAG CAC CGC A, as provided in SEQ ID NO: 197 and 198, respectively. PCR amplification of the gene under conditions well known in the art and ligation into the 5' ASCI/3' MluI sites of the JAL vector was completed after inserting the short nucleotide sequence GCAATC (SEQ ID NO: 199) upstream of the ATG to create a Kozak-like sequence. The resulting expression vector contained the full-length pmpC gene comprising 5325 nucleotides (SEQ ID NO: 173) containing the hypothetical signal sequence, which encodes a 187 kD protein (SEQ ID NO: 179). The pmpD gene was subcloned into the JA4304 vaccine vector following PCR amplification of the gene using the following oligos: 5' oligo- TGC AAT CAT GAG TTC GCA GAA AGA TAT AAA AAG C

(SEO ID NO: 200) and 3' oligo- CAG AGC TAG CTT AAA AGA TCA ATC GCA ATC CAG TAT TC (SEO ID-NO: 201). The gene was ligated into the a 5' blunted HIII/3' MluI site of the JA4304 vaccine vector using standard techniques well known in the art. The CAATC (SEQ ID NO: 202) was inserted upstream of the ATG to create a Kozak-like sequence. This clone is unique in that the last threonine of the HindIII site is missing due to the blunting procedure, as is the last glycine of the Kozak-like sequence. The insert, a 4593 nucleotide fragment (SEQ ID NO: 172) is the full-length gene for pmpD containing the hypothetical signal sequence, which encodes a 161 kD protein (SEQ ID NO: 178). PmpE was subcloned into the JA4304 vector using the 5' oligo- TGC AAT CAT GAA AAA AGC GTT TTT CTT TTT C (SEQ ID NO: 203), and the 3' oligo- CAG AAC GCG TCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 204). Following PCR amplification, the gene was ligated into the 5' blunted HIII/3' MluI site of JA4304. To facilitate this, a short nucleotide sequence, TGCAATC (SEQ ID NO: 293), was added upstream of the initiation codon for creating a Kozak-like sequence and reconstituting the HindIII site. The insert is the full-length pmpE gene (SEQ ID NO: 171) containing the hypothetical signal sequence. The pmpE gene encodes a 105 kD protein (SEQ ID NO: 177). The pmpG gene was PCR amplified using the 5' oligo- GTG CAA TCA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 205), and the 3' oligo- CAG AAC GCG TTT AGA ACC GGA CTT TAC TTC C (SEQ ID NO: 206) and subcloned into the JA4304 vector. Similar cloning strategies were followed for the pmpI and pmpK genes. In addition, primer pairs were designed to PCR amplify the full-length or overlapping fragments of the pmp genes, which were then subcloned for protein expression in the pET17b vector (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Several of the genes encoding the recombinant proteins, as described below, lack the native signal sequence to Full-length protein expression of pmpC was facilitate expression of the protein. accomplished through expression of two overlapping fragments, representing the amino and carboxy termini. Subcloning of the pmpC-amino terminal portion, which lacks the signal sequence, (SEQ ID NO: 187, with the corresponding amino acid sequence provided in SEQ ID NO: 195) used the 5' oligo- CAG ACA TAT GCA TCA CCA TCA CCA TCA CGA

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GGC GAG CTC GAT CCA AGA TC (SEQ ID NO: 207), and the 3' oligo- CAG AGG TAC CTC AGA TAG CAC TCT CTC CTA TTA AAG TAG G (SEQ ID-NO: 208) into the 5' NdeI/3' KPN cloning site of the vector. The carboxy terminus portion of the gene, pmpCcarboxy terminal fragment (SEQ ID NO: 186, with the corresponding amino acid sequence provided in SEQ ID NO: 194), was subcloned into the 5' NheI/3' KPN cloning site of the expression vector using the following primers: 5' oligo- CAG AGC TAG CAT GCA TCA CCA TCA CCA TCA CGT TAA GAT TGA GAA CTT CTC TGG C (SEQ ID NO: 209), and 3' oligo- CAG AGG TAC CTT AGA ATG TCA TAC GAG CAC CGC AG (SEQ ID NO: 210). PmpD was also expressed as two overlapping proteins. The pmpD-amino terminal portion, which lacks the signal sequence, (SEQ ID NO: 185, with the corresponding amino acid sequence provided in SEQ ID NO: 193) contains the initiating codon of the pET17b and is expressed as a 80 kD protein. For protein expression and purification purposes, a six-histidine tag follows the initiation codon and is fused at the 28th amino acid (nucleotide 84) of the gene. The following primers were used, 5' oligo, CAG ACA TAT GCA TCA CCA TCA CGG GTT AGC (SEQ ID NO: 211), and the 3' oligo-CAG AGG TAC CTC AGC TCC AGC ACA CTC TCT TC (SEQ ID NO: 212), to splice into the 5' NdeI/3' KPN cloning site of the vector. The pmpD-carboxy terminus portion (SEQ ID NO: 184) was expressed as a 92 kD protein (SEQ ID NO: 192). For expression and subsequent purification, an additional methionine, alanine and serine was included, which represent the initiation codon and the first two amino acids from the pET17b vector. A six-histidine tag downstream of the methionine, alanine and serine is fused at the 691st amino acid (nucleotide 2073) of the gene. The 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA CGG TGC TAT TTC TTG CTT ACG TGG (SEQ ID NO: 213) and the 3' oligo- CAG AGG TAC TTn AAA AGA TCA ATC GCA ATC CAG TAT TCG (SEQ ID NO: 214) were used to subclone the insert into the 5' NheI/3' KPN cloning site of the expression vector. PmpE was expressed as a 106kD protein (SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191). The pmpE insert also lacks the native signal sequence. PCR amplification of the gene under conditions well known in the art was performed using the following oligo primers: 5' oligo- CAG AGG ATC CAC ATC ACC ATC ACC ATC ACG GAC TAG CTA GAG AGG TTC (SEQ ID NO: 215), and

the 3' oligo- CAG AGA ATT CCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 216), and the amplified insert was ligated into a 5' BamHI/3' EcoRI site of JA4304. The short nucleotide sequence, as provided in SEQ ID NO: 217, was inserted upstream of the initiation codon for creating the Kozak-like sequence and reconstituting the HindIII site. expressed protein contains the initiation codon and the downstream 21 amino acids from the pET17b expression vector, i.e., MASMTGGQQMGRDSSLVPSSDP (SEQ ID NO: 218). In addition, a six-histidine tag is included upstream of the sequence described above and is fused at the 28th amino acid (nucleotide 84) of the gene, which eliminates the hypothetical signal peptide. The sequences provided in SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191 do not include these additional sequences. The pmpG gene (SEQ ID NO: 182, with the corresponding amino acid sequence provided in SEQ ID No; 190) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo- CAG AGG TAC CGC ATC ACC ATC ACC ATC ACA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 219), and the 3' oligo- CAG AGC GGC CGC TTA GAA CCG GAC TTT ACT TCC (SEQ ID NO: 220), and ligated into the 5' KPN/3' NotI cloning site of the expression vector. The expressed protein contains an additional amino acid sequence at the amino end, namely, MASMTGGQQNGRDSSLVPHHHHHHH (SEQ ID NO: 221), which comprises the initiation codon and additional sequence from the pET17b expression vector. The pmpI gene (SEQ ID NO: 181, with the corresponding amino acid sequence provided in SEQ ID No; 189) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA CCT CTT TGG CCA GGA TCC C (SEQ ID NO: 222), and the 3' oligo- CAG AAC TAG TCT AGA ACC TGT AAG TGG TCC (SEQ ID NO: 223), and ligted into the expression vector at the 5' NheI/3' SpeI cloning site. The 95 kD expressed protein contains the initiation codon plus an additional alanine and serine from the pET17b vector at the amino end of the protein. In addition, a six-histidine tag is fused at the 21st amino acid of the gene, which eliminates the hypothetical signal peptide.

Clone 14H1-4, (SEQ ID NO: 56), identified using the TCT-3 cell line, contains a complete ORF for the TSA gene, thiol specific antioxidant — CT603 (the CT603 ORF is a homolog of CPn0778 from *C. pnuemoniae*). The TSA open reading frame in clone

14-H1-4 was amplified such that the expressed protein possess an additional methionine and a 6x histidine tag (amino terminal end). This amplified insert was sub-cloned into the Nde/EcoRI sites of the pET17b vector. Upon induction of this clone with IPTG, a 22.6 kDa protein was purified by Ni-NTA agarose affinity chromatography. The determined amino acid sequence for the 195 amino acid ORF of clone 14-H1-4 encoding the TSA gene is provided in SEQ ID NO: 65. Further analysis yielded a full-length clone for the TSA gene, referred to as CTL2-TSA-FL, with the full-length amino acid sequence provided in SEQ ID NO: 92.

Further studies yielded 10 additional clones identified by the TCT-1 and TCT-3 T-cell lines, as described above. The clones identified by the TCT-1 line are: 16-D4-22, 17-C5-19, 18-C5-2, 20-G3-45 and 21-C7-66; clones identified by the TCT-3 cell line are: 17-C10-31, 17-E2-9, 22-A1-49 and 22-B3-53. Clone 21-G12-60 was recognized by both the TCT-1 and TCT-3 T cell lines. Clone 16-D4-22 (SEQ ID NO: 119), identified using the TCT-1 cell line contains a 953 bp insert that contains two genes, parts of open reading frame 3 (ORF3) and ORF4 of the C. trachomatis plasmid for growth within mammalian cells. Clone 17-C5-19 (SEQ ID NO: 118), contains a 951 bp insert that contains part of the ORF for DT431, encoding for clpP_1 protease and part of the ORF for CT430 (diaminopimelate epimerase). Clone 18-C5-2 (SEQ ID NO: 117) is part of the ORF for S1 ribosomal protein with a 446 bp insert that was identified using the TCT-1 cell line. Clone 20-G3-45 (SEQ ID NO: 116), identified by the TCT-1 cell line, contains a 437 bp insert that is part of the pmpB gene (CT413). Clone 21-C7-66 (SEQ ID NO: 115), identified by the TCT-1 line, contains a 995bp insert that encodes part of the dnaK like protein. The insert of this clone does not overlap with the insert of the TCT-3 clone 11-H4-28 (SEQ ID NO: 59), which was shown to be part of the dnaK gene CT396 Clone 17-C10-31 (SEQ ID NO: 114), identified by the TCT-3 cell line, contains a 976 bp insert. This clone contains part of the ORF for CT858, a protease containing IRBP and DHR domains. Clone 17-E2-9 (SEQ ID NO: 113) contains part of ORFs for two genes, CT611 and CT610, that span a 1142 bp insert. Clone 22-A1-49 (SEQ ID NO: 112), identified using the TCT-3 line, also contains two genes in a 698 bp insert. Part of the ORF for CT660 (DNA gyrase{gyrA_2}) is present on the top strand where as the complete ORF for a hypothetical protein CT659 is present on the complementary

strand. Clone 22-B3-53 (SEQ ID NO: 111), identified by the TCT-1 line, has a 267 bp insert that encodes part of the ORF for GroEL (CT110). Clone 21-G12-60 (SEQ ID NO: 110), identified by both the TCT-1 and TCT-3 cell lines contains a 1461 bp insert that contains partial ORFs for hypothetical proteins CT875, CT229 and CT228.

Additional Chlamydia antigens were obtained by screening a genomic expression library of Chlamydia trachomatis (LGV II serovar) in Lambda Screen-1 vector (Novagen, Madison, WI) with sera pooled from several Chlamydia-infected individuals using techniques well known in the art. The following immuno-reactive clones were identified and the inserts containing Chlamydia genes sequenced: CTL2#1 (SEQ ID NO: 71); CTL2#2 (SEQ ID NO: 70); CTL2#3-5' (SEQ ID NO: 72, a first determined genomic sequence representing the 5' end); CTL2#3-3' (SEQ ID NO: 73, a second determined genomic sequence representing the 3' end); CTL2#4 (SEQ ID NO: 53); CTL2#5 (SEQ ID NO: 69); CTL2#6 (SEQ ID NO: 68); CTL2#7 (SEQ ID NO: 67); CTL2#8b (SEQ ID NO: 54); CTL2#9 (SEQ ID NO: 66); CTL2#10-5' (SEQ ID NO: 74, a first determined genomic sequence representing the 5' end); CTL2#10-3' (SEQ ID NO: 75, a second determined genomic sequence representing the 3' end); CTL2#11-5' (SEQ ID NO: 45, a first determined genomic sequence representing the 5' end); CTL2#11-3' (SEQ ID NO: 44, a second determined genomic sequence representing the 3' end); CTL2#12 (SEQ ID NO: 46); CTL2#16-5' (SEQ ID NO: 47); CTL2#18-5' (SEQ ID NO: 49, a first determined genomic sequence representing the 5' end); CTL2#18-3' (SEQ ID NO: 48, a second determined genomic sequence representing the 3' end); CTL2#19-5' (SEQ ID NO: 76, the determined genomic sequence representing the 5' end); CTL2#21 (SEQ ID NO: 50); CTL2#23 (SEQ ID NO: 51; and CTL2#24 (SEQ ID NO: 52).

Additional *Chlamydia trachomatis* antigens were identified by serological expression cloning. These studies used sera pooled from several *Chlamydia*-infected individuals, as described above, but, IgA, and IgM antibodies were used in addition to IgG as a secondary antibody. Clones screened by this method enhance detection of antigens recognized by an early immune response to a *Chlamydial* infection, that is a mucosal humoral immune response. The following immunoreactive clones were characterized and the inserts containing *Chlamydia* genes sequenced: CTL2gam-1 (SEQ ID NO: 290), CTL2gam-2 (SEQ

ID NO: 289), CTL2gam-5 (SEQ ID NO: 288), CTL2gam-6-3' (SEQ ID NO: 287, a second determined genomic sequence representing the 3' end), CTL2gam-6-5' (SEQ ID NO: 286, a first determined genomic sequence representing the 5' end), CTL2gam-8 (SEQ ID NO: 285), CTL2gam-10 (SEQ ID NO: 284), CTL2gam-13 (SEQ ID NO: 283), CTL2gam-15-3' (SEQ ID NO: 282, a second determined genomic sequence representing the 3' end), CTL2gam-15-5' (SEQ ID NO: 281, a first determined genomic sequence representing the 5' end), CTL2gam-17 (SEQ ID NO: 280), CTL2gam-18 (SEQ ID NO: 279), CTL2gam-21 (SEQ ID NO: 278), CTL2gam-23 (SEQ ID NO: 277), CTL2gam-24 (SEQ ID NO: 276), CTL2gam-26 (SEQ ID NO: 275), CTL2gam-27 (SEQ ID NO: 274), CTL2gam-28 (SEQ ID NO: 273), CTL2gam-30-3' (SEQ ID NO: 272, a second determined genomic sequence representing the 3' end) and CTL2gam-30-5' (SEQ ID NO: 271, a first determined genomic sequence representing the 5' end).

EXAMPLE 2

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y PRODUCTION BY CHLAMYDIA TRACHOMATIS ANTIGENS

The ability of recombinant *Chlamydia trachomatis* antigens to induce T cell proliferation and interferon-γ production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatograph (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. trachomatis* patients as well as from normal donors whose T-cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μg/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 μ g/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μl, 50 μl of medium is removed from each well for determination of IFN-γ levels, as described below. The plates are then pulsed with 1 μCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that

result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ is measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

Using the above methodology, recombinant 1B1-66 protein (SEQ ID NO: 5) as well as two synthetic peptides corresponding to amino acid residues 48-67 (SEQ ID NO: 13; referred to as 1-B1-66/48-67) and 58-77 (SEQ ID NO: 14, referred to as 1B1-66/58-77), respectively, of SEQ ID NO: 5, were found to induce a proliferative response and IFN-γ production in a Chlamydia-specific T cell line used to screen a genomic library of C. trachomatis LGV II.

Further studies have identified a *C. trachomatis*-specific T-cell epitope in the ribosomal S13 protein. Employing standard epitope mapping techniques well known in the art, two T-cell epitopes in the ribosomal S13 protein (rS13) were identified with a *Chlamydia*-specific T-cell line from donor CL-8 (T-cell line TCL-8 EB/DC). Fig. 8 illustrates that the first peptide, rS13 1-20 (SEQ ID NO: 106), is 100% identical with the corresponding *C. pneumoniae* sequence, explaining the cross-reactivity of the T-cell line to recombinant *C. trachomatis*- and *C. pneumoniae*-rS13. The response to the second peptide

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rS13 56-75 (SEQ ID NO: 108) is *C. trachomatis*-specific, indicating that the rS13 response in this healthy asymptomatic donor was elicited by exposure to *C. trachomatis* and not to *C. pneumoniae*, or any other microbial infection.

As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10⁴ TCP-21 T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-21 T-cell line also gave a proliferative response to the homologous C. pneumoniae peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the C. trachomatis peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between C. trachomatis and C. pneumoniae.

To further define the epitope described above, an additional T-cell line, TCT-3, was used in epitope mapping experiments. The immunoassays were performed as described above, except that only peptides from *C. trachomatis* were tested. The T-cells gave a proliferative response to two peptides, CT-OMCB #152-171 and CT-OMCB #157-176 (SEQ ID NO: 246 and 247, respectively), thereby defining an additional immunogenic epitope in the cysteine rich outer membrane protein of *C. trachomatis*.

Clone 14H1-4, (SEQ ID NO: 56, with the corresponding full-length amino acid sequence provided in SEQ ID NO: 92), was identified using the TCT-3 cell line in the CD4 T-cell expression cloning system previously described, and was shown to contain a complete ORF for the, thiol specific antioxidant gene (CT603), referred to as TSA. Epitope

mapping immunoassays were performed, as described above, to further define the epitope. The TCT-3 T-cells line exhibited a strong proliferative response to the overlapping peptides CT-TSA #96-115, CT-TSA #101-120 and CT-TSA #106-125 (SEQ ID NO: 254-256, respectively) demonstrating an immunoreactive epitope in the thiol specific antioxidant gene of *C. trachomatis* serovar LGVII.

EXAMPLE 3 PREPARATION OF SYNTHETIC POLYPEPTIDES

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugating or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray mass spectrometry and by amino acid analysis.

EXAMPLE 4

ISOLATION AND CHARACTERIZATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS USING RETROVIRAL EXPRESSION VECTOR SYSTEMS AND SUBSEQUENT IMMUNOLOGICAL ANALYSIS

A genomic library of *Chlamydia trachomatis* LGV II was constructed by limited digests using BamHI, BgIII, BstYi and MboI restriction enzymes. The restriction digest fragments were subsequently ligated into the BamHI site of the retroviral vectors pBIB-KS1,2,3. This vector set was modified to contain a Kosak translation initiation site and stop codons in order to allow expression of proteins from short DNA genomic fragments, as shown in Fig. 2. DNA pools of 80 clones were prepared and transfected into the retroviral packaging line Phoenix-Ampho, as described in Pear, W.S., Scott, M.L. and Nolan, G.P., Generation of High Titre, Helper-free Retroviruses by Transient Transfection. Methods in Molecular Medicine: Gene Therapy Protocols, Humana Press, Totowa, NJ, pp. 41-57. The *Chlamydia* library in retroviral form was then transduced into H2-Ld expressing P815 cells, which were then used as target cells to stimulate an antigen specific T-cell line.

A Chlamydia-specific, murine H2^d restricted CD8+ T-cell line was expanded in culture by repeated rounds of stimulation with irradiated C. trachomatis-infected J774 cells and irradiated syngeneic spleen cells, as described by Starnbach, M., in J. Immunol., 153:5183, 1994. This Chlamydia-specific T-cell line was used to screen the above Chlamydia genomic library expressed by the retrovirally-transduced P815 cells. Positive DNA pools were identified by detection of IFN-γ production using Elispot analysis (SEE Lalvani et al., J. Experimental Medicine 186:859-865, 1997).

Two positive pools, referred to as 2C7 and 2E10, were identified by IFN-γ Elispot assays. Stable transductants of P815 cells from pool 2C7 were cloned by limiting dilution and individual clones were selected based upon their capacity to elicit IFN-γ production from the *Chlamydia*-specific CTL line. From this screening process, four positive clones were selected, referred to as 2C7-8, 2C7-9, 2C7-19 and 2C7-21. Similarly, the positive pool 2E10 was further screened, resulting in a an additional positive clone, which

contains three inserts. The three inserts are fragments of the CT016, tRNA syntase and clpX genes (SEQ ID NO: 268-270, respectively).

Transgenic DNA from these four positive 2C7.8 clones were PCR amplified using pBIB-KS specific primers to selectively amplify the *Chlamydia* DNA insert. Amplified inserts were gel purified and sequenced. One immunoreactive clone, 2C7-8 (SEQ ID NO: 15, with the predicted amino acid sequence provided in SEQ ID NO: 32), is a 160 bp fragment with homology to nucleotides 597304-597145 of *Chlamydia trachomatis*, serovar D (NCBI, BLASTN search; SEQ ID NO: 33, with the predicted amino acid sequence provided in SEQ ID NO: 34). The sequence of clone 2C7-8 maps within two putative open reading frames from the region of high homology described immediately above, and in particular, one of these putative open reading frames, consisting of a 298 amino acid fragment (SEQ ID NO: 16, with the predicted amino acid sequence provided in SEQ ID NO: 17), was demonstrated to exhibit immunological activity.

Full-length cloning of the 298 amino acid fragment (referred to as CT529 and/or the Capl gene) from serovar L2 was obtained by PCR amplification using 5'-ttttgaagcaggtaggtgaatatg (forward) (SEQ ID NO: 159) and 5'-ttaagaaatttaaaaaatccctta (reverse) (SEQ ID NO: 160) primers, using purified *C. trachomatis* L2 genomic DNA as template. This PCR product was gel-purified, cloned into pCRBlunt (Invitrogen, Carlsbad, CA) for sequencing, and then subcloned into the *Eco*RI site of pBIB-KMS, a derivative of pBIB-KS for expression. The *Chlamydia pnuemoniae* homlogue of CT529 is provided in SEQ ID NO: 291, with the corresponding amino acid sequence provided in SEQ ID NO: 292.

Full-length DNA encoding various CT529 serovars were amplified by PCR from bacterial lysates containing 10⁵ IFU, essentially as described (Denamur, E., C. Sayada, A. Souriau, J. Orfila, A. Rodolakis and J. Elion. 1991. J. Gen. Microbiol. 137: 2525). The following serovars were amplified as described: Ba (SEQ ID NO: 134, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 135); E (BOUR) and E (MTW447) (SEQ ID NO: 122, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 123); F (NI1) (SEQ ID NO: 128, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 126, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 127); Ia (SEO ID

NO: 124, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 125); L1 (SEQ ID NO: 130, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 131); L3 (SEQ ID NO: 132, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 133); I (SEQ ID NO: 263, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 264); K (SEQ ID NO: 265, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 266); and MoPn (SEQ ID NO: 136, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 137). PCR reactions were performed with Advantage Genomic PCR Kit (Clontech, Palo Alto, CA) using primers specific for serovar L2 DNA (external to the ORF). Primers sequences were 5'-ggtataatatctctctaaattttg (forward-SEQ ID NO: 161) and 5'-agataaaaaaaggctgtttc' (reverse-SEQ ID NO: 162) except for MoPn which required 5'-ttttgaagcaggtaggtgaatatg (forward-SEQ ID NO: 163) and 5'-tttacaataagaaaagctaagcactttgt (reverse-SEQ ID NO: 164). PCR amplified DNA was purified with QIAquick PCR purification kit (Qiagen, Valencia, CA) and cloned in pCR2.1 (Invitrogen, Carlsbad, CA) for sequencing.

Sequencing of DNA derived from PCR amplified inserts of immunoreactive clones was done on an automated sequencer (ABI 377) using both a pBIB-KS specific forward primer 5'-ccttacacagtcctgctgac (SEQ ID NO: 165) and a reverse primer 3'-gtttccgggccctcacattg (SEQ ID NO: 166). PCRBlunt cloned DNA coding for CT529 serovar L2 and pCR2.1 cloned DNA coding for CT529 serovar Ba, E (BOUR), E (MTW447), F (NI1), G, Ia, K, L1, L3 and MoPn were sequenced using T7 promoter primer and universal M13 forward and M13 reverse primers.

To determine if these two putative open reading frames (SEQ ID NO: 16 and 20) encoded a protein with an associated immunological function, overlapping peptides (17-20 amino acid lengths) spanning the lengths of the two open reading frames were synthesized, as described in Example 3. A standard chromium release assay was utilized to determine the per cent specific lysis of peptide-pulsed H2^d restricted target cells. In this assay, aliquots of P815 cells (H2^d) were labeled at 37° C for one hour with 100 μ Ci of ⁵¹Cr in the presence or absence of 1 μ g/ml of the indicated peptides. Following this incubation, labeled P815 cells were washed to remove excess ⁵¹Cr and peptide, and subsequently plated

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in duplicate in microculture plates at a concentration of 1,000 cells/well. Effector CTL (Chlamydia-specific CD8 T cells) were added at the indicated effector:target ratios. Following a 4 hour incubation, supernatants were harvested and measured by gamma-counter for release of ⁵¹Cr into the supernatant. Two overlapping peptides from the 298 amino acid open reading frame did specifically stimulate the CTL line. The peptides represented in SEQ ID NO: 138-156 were synthsized, representing the translation of the L2 homologue of the serovar D open reading frame for CT529 (Cap1 gene) and 216 amino acid open reading frame.As shown in Fig. 3, peptides CtC7.8-12 (SEQ ID NO: 18, also referred to as Cap1#132-147, SEQ ID NO: 139) and CtC7.8-13 (SEQ ID NO: 19, also referred to as Cap1#138-155, SEQ ID NO: 140) were able to elicit 38 to 52% specific lysis, respectively, at an effector to target ratio of 10:1. Notably, the overlap between these two peptides contained a predicted H2^d (K^d and L^d) binding peptide. A 10 amino acid peptide was synthesized to correspond to this overlapping sequence (SEQ ID NO: 31) and was found to generate a strong immune response from the anti-Chlamydia CTL line by elispot assay. Significantly, a search of the most recent Genbank database revealed no proteins have previously been described for this gene. Therefore, the putative open reading frame encoding clone 2C7-8 (SEQ ID NO: 15) defines a gene which encompasses an antigen from Chlamydia capable of stimulating antigen-specific CD8+ T-cells in a MHC-I restricted manner, demonstrating this antigen could be used to develop a vaccine against Chlamydia.

To confirm these results and to further map the epitope, truncated peptides (SEQ ID NO: 138-156) were made and tested for recognition by the T-cells in an IFN-g ELISPOT assay. Truncations of either Ser139 (Cap1#140-147, SEQ ID NO: 146) or Leu147 (Cap1#138-146, SEQ ID NO: 147) abrogate T-cell recognition. These results indicate that the 9-mer peptide Cap1#139-147 (SFIGGITYL, SEQ ID NO: 145) is the minimal epitope recognized by the *Chlamydia*-specific T-cells.

Sequence alignments of Cap1 (CT529) from selected serovars of *C. trachomatis* (SEQ ID NO: 121, 123, 125, 127, 129, 131, 133, 135, 137 and 139) shows one of the amino acid differences is found in position 2 of the proposed epitope. The homologous serovar D peptide is SIIGGITYL (SEQ ID NO: 168). The ability of SFIGGITYL and SIIGGITYL to target cells for recognition by the *Chlamydia* specific T-cells was compared.

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Serial dilutions of each peptide were incubated with P815 cells and tested for recognition by the T-cells in a ⁵¹Cr release assay, as described above. The *Chlamydia*-specific T-cells recognize the serovar L2 peptide at a minimum concentration of 1 nM and the serovar D peptide at a minimum concentration of 10 nM.

Further studies have shown that a Cap1#139-147-specific T-cell clone recognizes *C. trachomatis* infected cells. To confirm that Cap1₁₃₉₋₁₄₇ is presented on the surface of *Chlamydia* infected cells, Balb-3T3 (H-2^d) cells were infected with *C. trachomatis* serovar L2 and tested to determine whether these cells are recognized by a CD8+ T-cell clone specific for Cap1#139-147 epitope (SEQ ID NO: 145). The T-cell clone specific for Cap1#139-147 epitope was obtained by limiting dilution of the line 69 T-cells. The T-cell clone specifically recognized the *Chlamydia* infected cells. In these experiments, target cells were *C. trachomatis* infected (positive control) or uninfected Balb/3T3 cells, showing 45%, 36% and 30% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively; or Cap1#139-147 epitope (SEQ ID NO: 145) coated, or untreated P815 cells, showing 83%, 75% and 58% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively (negative controls having less than 5% lysis in all cases). This data suggests that the epitope is presented during infection.

In vivo studies show Cap1#139-147 epitope-specific T-cells are primed during murine infection with *C. trachomatis*. To determine if infection with *C. trachomatis* primes a Cap1#139-147 epitope-specific T-cell response, mice were infected i.p. with 10⁸ IFU of *C. trachomatis* serovar L2. Two weeks after infection, the mice were sacrificed and spleen cells were stimulated on irradiated syngeneic spleen cells pulsed with Cap1#139-147 epitope peptide. After 5 days of stimulation, the cultures were used in a standard ⁵¹Cr release assay to determine if there were Cap1#139-147 epitope-specific T-cells present in the culture. Specifically, spleen cells from a *C. trachomatis* serovar L2 immunized mouse or a control mouse injected with PBS after a 5 days culture with Cap1#139-147 peptide-coated syngeneic spleen cells and CD8+ T-cells able to specifically recognize Cap1#139-147 epitope gave 73%, 60% and 32% specific lysis at a30:1, 10:1 and 3:1 effector to target ratios, respectively. The control mice had a percent lysis of approximately 10% at a 30:1 effector to target ratio, and steadily declining with lowering E:T ratios. Target cells were Cap1#139-147 peptide-

coated, or untreated P815 cells. These data suggest that Cap1#139-147 peptide-specific T-cells are primed during murine infection with *C. trachomatis*.

EXAMPLE 5

GENERATION OF ANTIBODY AND T-CELL RESPONSES IN MICE IMMUNIZED WITH CHLAMYDIA ANTIGENS

Immunogenicity studies were conducted to determine the antibody and CD4+ T cell responses in mice immunized with either purified SWIB or S13 proteins formulated with Montanide adjuvant, or DNA-based immunizations with pcDNA-3 expression vectors containing the DNA sequences for SWIB or S13. SWIB is also referred to as clone 1-B1-66 (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5), and S13 ribosomal protein is also referred to as clone 10-C10-31 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12). In the first experiment, groups of three C57BL/6 mice were immunized twice and monitored for antibody and CD4+ DNA immunizations were intradermal at the base of the tail and T-cell responses. polypeptide immunizations were administered by subcutaneous route. Results from standard ³H-incorporation assays of spleen cells from immunized mice shows a strong proliferative response from the group immunized with purified recombinant SWIB polypeptide (SEQ ID NO: 5). Further analysis by cytokine induction assays, as previously described, demonstrated that the group immunized with SWIB polypeptide produced a measurable IFN-y and IL-4 response. Subsequent ELISA-based assays to determine the predominant antibody isotype response in the experimental group immunized with the SWIB polypeptide were performed. Fig. 4 illustrates the SWIB-immunized group gave a humoral response that was predominantly IgG1.

In a second experiment, C3H mice were immunized three times with $10~\mu g$ purified SWIB protein (also referred to as clone 1-B1-66, SEQ ID NO: 5) formulated in either PBS or Montanide at three week intervals and harvested two weeks after the third immunization. Antibody titers directed against the SWIB protein were determined by

standard ELISA-based techniques well known in the art, demonstrating the SWIB protein formulated with Montanide adjuvant induced a strong humoral immune response. T-cell proliferative responses were determined by a XTT-based assay (Scudiero, et al, *Cancer Research*, 1988, 48:4827). As shown in Fig. 5, splenocytes from mice immunized with the SWIB polypeptide plus Montanide elicited an antigen specific proliferative response. In addition, the capacity of splenocytes from immunized animals to secrete IFN-γ in response to soluble recombinant SWIB polypeptide was determined using the cytokine induction assay previously described. The splenocytes from all animals in the group immunized with SWIB polypeptide formulated with montanide adjuvant secreted IFN-γ in response to exposure to the SWIB Chlamydia antigen, demonstrating an *Chlamydia*-specific immune response.

In a further experiment, C3H mice were immunized at three separate time points at the base of the tail with 10 μg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) formulated with the SBAS2 adjuvant (SmithKline Beecham, London, England). Antigen-specific antibody titers were measured by ELISA, showing both polypeptides induced a strong IgG response, ranging in titers from 1 x10⁻⁴ to 1 x10⁻⁵. The IgG1 and IgG2a components of this response were present in fairly equal amounts. Antigen-specific T-cell proliferative responses, determined by standard ³H-incorporation assays on spleen cells isolated from immunized mice, were quite strong for SWIB (50,000 cpm above the negative control) and even stronger for s13 (100,000 cpm above the negative control). The IFNγ production was assayed by standard ELISA techniques from supernatant from the proliferating culture. *In vitro* restimulation of the culture with S13 protein induced high levels of IFNγ production, approximately 25 ng/ml versus 2 ng/ml for the negative control. Restimulation with the SWIB protein also induced IFNγ, although to a lesser extent.

In a related experiment, C3H mice were immunized at three separate time points with 10 µg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) mixed with 10 µg of Cholera Toxin. Mucosal immunization was through intranasal inoculation. Antigenspecific antibody responses were determined by standard ELISA techniques. Antigenspecific IgG antibodies were present in the blood of SWIB-immunized mice, with titers

ranging from 1 x10⁻³ to 1 x10⁻⁴, but non-detectable in the S13-immunized animals. Antigen-specific T-cell responses from isolated splenocytes, as measured by IFNγ production, gave similar results to those described immediately above for systemic immunization.

An animal study was conducted to determine the immunogenicity of the CT529 serovar LGVII CTL epitope, defined by the CT529 10mer consensus peptide (CSFIGGITYL - SEQ ID NO: 31), which was identified as an H2-Kd restricted CTL epitope. BALB/c mice (3 mice per group) were immunized three times with 25 µg of peptide combined with various adjuvants. The peptide was administered systemically at the base of the tail in either SKB Adjuvant System SBAS-2", SBAS-7 (SmithKline Beecham, London, England) or Montanide. The peptide was also administered intranasally mixed with 10ug of Cholera Toxin (CT). Naive mice were used as a control. Four weeks after the 3rd immunization, spleen cells were restimulated with LPS-blasts pulsed with 10ug/ml CT529 10mer consensus peptide at three different effector to LPS-blasts ratios: 6, 1.5 and 0.4 at 1x106 cell/ml. After 2 restimulations, effector cells were tested for their ability to lyse peptide pulsed P815 cells using a standard chromium release assay. A non-relevant peptide from chicken egg ovalbumin was used as a negative control. The results demonstrate that a significant immune response was elicited towards the CT529 10mer consensus peptide and that antigen-specific T-cells capable of lysing peptide-pulsed targets were elicited in response to immunization with the peptide. Specifically, antigen-specific lytic activities were found in the SBAS-7 and CT adjuvanted group while Montanide and SBAS-2" failed to adjuvant the CTL epitope immunization.

EXAMPLE 6

EXPRESSION AND CHARACTERIZATION OF CHLAMYDIA PNEUMONIAE GENES

The human T-cell line, TCL-8, described in Example 1, recognizes *Chlamydia trachomatis* as well as *Chlamydia pneumonia* infected monocyte-derived dendritic cells, suggesting *Chlamydia trachomatis* and *pneumonia* may encode cross-reactive T-cell epitopes. To isolate the *Chlamydia pneumonia* genes homologous to *Chlamydia trachomatis* LGV II

clones 1B1-66, also referred to as SWIB (SEQ ID NO: 1) and clone 10C10-31, also referred to as S13 ribosomal protein (SEQ ID NO: 4), HeLa 229 cells were infected with C pneumonia strain TWAR (CDC/CWL-029). After three days incubation, the C pneumonia-infected HeLa cells were harvested, washed and resuspended in 200 μ l water and heated in a boiling water bath for 20 minutes. Ten microliters of the disrupted cell suspension was used as the PCR template.

C. pneumonia specific primers were designed for clones 1B1-66 and 10C10-31 such that the 5' end had a 6X-Histidine tag and a Nde I site inserted, and the 3' end had a stop codon and a BamHI site included (Fig. 6). The PCR products were amplified and sequenced by standard techniques well known in the art. The C. pneumonia-specific PCR products were cloned into expression vector pET17B (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Two proteins from C. pneumonia were thus generated, a 10-11 kDa protein referred to as CpSWIB (SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 30 and 91, respectively).

EXAMPLE 7

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y PRODUCTION BY CHLAMYDIA PNEUMONIAE ANTIGENS

The ability of recombinant *Chlamydia pneumoniae* antigens to induce T cell proliferation and interferon-γ production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatography (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. pneumoniae* patients as well as from normal donors whose T-

cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μ g/ml-gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 μ g/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μ l, 50 μ l of medium is removed from each well for determination of IFN- γ levels, as described below. The plates are then pulsed with 1 μ Ci/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

A human anti-Chlamydia T-cell line (TCL-8) capable of cross-reacting to C. trachomatis and C. pneumonia was used to determine whether the expressed proteins described in the example above, (i.e., CpSWIB, SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 28, respectively, and the 15 kDa protein referred to as CpS13 SEQ ID NO: 29, and SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID

NO: 30 and 91, respectively), possessed T-cell epitopes common to both C. trachomatis and C. pneumonia. Briefly, E. coli expressing Chlamydial proteins were titered on 1 x 104 monocyte-derived dendritic cells. After two hours, the dendritic_cells cultures were washed and 2.5×10^4 T cells (TCL-8) added and allowed to incubate for an additional 72 hours. The amount of INF- γ in the culture supernatant was then determined by ELISA. As shown in Figs. 7A and 7B, the TCL-8 T-cell line specifically recognized the S13 ribosomal protein from both C. trachomatis and C. pneumonia as demonstrated by the antigen-specific induction of IFN-7, whereas only the SWIB protein from C. trachomatis was recognized by the T-cell line. To validate these results, the T cell epitope of C. trachomatis SWIB was identified by epitope mapping using target cells pulsed with a series of overlapping peptides and the T-cell line TCL-8. 3H-thymidine incorporation assays demonstrated that the peptide, referred to as C.t.SWIB 52-67, of SEQ ID NO: 39 gave the strongest proliferation of the The homologous peptides corresponding to the SWIB of C. pneumoniae sequence (SEQ ID NO: 40), the topoisomerase-SWIB fusion of C. pneumoniae (SEQ ID NO: 43) and C. trachomatis (SEQ ID NO: 42) as well as the human SWI domain (SEQ ID NO: 41) were synthesized and tested in the above assay. The T-cell line TCL-8 only recognized the C. trachomatis peptide of SEQ ID NO: 39 and not the corresponding C. pneumoniae peptide (SEQ ID NO: 40), or the other corresponding peptides described above (SEQ ID NO; 41-43).

Chlamydia-specific T cell lines were generated from donor CP-21 with a positive serum titer against *C. pneumoniae* by stimulating donor PBMC with either *C. trachomatis* or *C. pneumoniae*-infected monocyte-derived dendritic cells, respectively. T-cells generated against *C. pneumoniae* responded to recombinant *C. pneumoniae*-SWIB but not *C. trachomatis*-SWIB, whereas the T-cell line generated against *C. trachomatis* did not respond to either *C. trachomatis*- or *C. pneumoniae*-SWIB (see Fig. 9). The *C. pneumoniae*-SWIB specific immune response of donor CP-21 confirms the *C. pneumoniae* infection and indicates the elicitation of *C. pneumoniae*-SWIB specific T-cells during *in vivo C. pneumoniae* infection.

Epitope mapping of the T-cell response to *C. pneumoniae*-SWIB has shown that Cp-SWIB-specific T-cells responded to the overlapping peptides Cp-SWIB 32-51 (SEQ

ID NO: 101) and Cp-SWIB 37-56 (SEQ ID NO: 102), indicating a *C. pneumoniae*-SWIB-specific T-cell epitope Cp-SWIB 37-51 (SEQ ID NO: 100).

In additional experiments, T-cell lines were generated from donor CP1, also a C. pneumoniae seropositive donor, by stimulating PBMC with non-infectious elementary bodies from C. trachomatis and C. pneumoniae, respectively. In particular, proliferative responses were determined by stimulating 2.5 x 10⁴ T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells and non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or either recombinant C. trachomatis or C. pneumoniae SWIB protein. The T-cell response against SWIB resembled the data obtained with T-cell lines from CP-21 in that C. pneumoniae-SWIB, but not C. trachomatis-SWIB elicited a response by the C. pneumoniae T-cell line. In addition, the C. trachomatis T-cell line did not proliferate in response to either C. trachomatis or C. pneumoniae SWIB, though it did proliferate in response to both CT and CP elementary bodies. As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10⁴ TCP-21 T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-21 T-cell line also gave a proliferative response to the homologous C. pneumoniae peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the to the C. trachomatis peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between C. trachomatis and C. pneumoniae.

EXAMPLE 8

IMMUNE RESPONSES OF HUMAN PBMC AND T-CELL LINES AGAINST CHLAMYDIA ANTIGENS

The examples provided herein suggest that there is a population of healthy donors among the general population that have been infected with C. trachomatis and generated a protective immune response controlling the C. trachomatis infection. These donors remained clinically asymptomatic and seronegative for C. trachomatis. To characterize the immune responses of normal donors against chlamydial antigens which had been identified by CD4 expression cloning, PBMC obtained from 12 healthy donors were tested against a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and C. trachomatis-, C. pneumoniae-S13. The data are summarized in Table I below. All donors were seronegative for C. trachomatis, whereas 6/12 had a positive C. pneumoniae titer. Using a stimulation index of >4 as a positive response, 11/12 of the subjects responded to C. trachomatis elementary bodies and 12/12 responded to C. One donor, AD104, responded to recombinant C. pneumoniae elementary bodies. pneumoniae-S13 protein, but not to recombinant C. trachomatis-S13 protein, indicating a C. pneumoniae-specific response. Three out of 12 donors had a C. trachomatis-SWIB, but not a C. pneumoniae-SWIB specific response, confirming a C. trachomatis infection. C. trachomatis and C. pneumoniae- S13 elicited a response in 8/12 donors suggesting a chlamydial infection. These data demonstrate the ability of SWIB and S13 to elicit a T-cell response in PBMC of normal study subjects.

Table I.

Immune response of normal	study subjects against Chlamadia
TITLE OF THE PARTY	SHAY SUCIOUS GEGILDI CHUMMIT

onor	Sex	<i>Chlamydia</i> IgGtiter	Cl EB	CP EB	CT Swib	CP Swib	CI S13	CP S13	CT lpdA	CT TSA
D100	male	negative	++	+++	+	-	++	++	-	nt.
D104	female	negative	+++	++	-	-	-	++	-	nt.
D108	male	CP 1:256	++	++	+	+/-	+	+	+	nt.
D112	female	negative	++	++	+	, -	+	-	+/-	nt.
D120	male	negative	-	+	-	-	-	-	-	n.t.
D124	female	CP 1:128	++-	++	-	-	-	-	-	nt.
D128	male	CP 1:512	+	++	-	-	++	+	++	-
D132	female	negative	++-	++	-	-	+	+	-	-
D136	female	CP 1:128	+	++	-	-	+/-	-	-	-
D140	male	CP 1:256	++-	++	-	-	+	+	-	-
D142	female	CP 1:512	++-	++-	-	-	+	+	+	-
D146	female	negative	++	++	-	-	++	+	+	-

CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant Chlamydia TSA protein. Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10⁵ PBMC with 1 x 10⁴ monocyte-derived dendritic cells pre-incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a ³H-thymidine pulse for the last 18h.

SI: Stimulation index

+/-:	SI ~	4
+:	SI>	4
++:	SI	10-30
+++:	SI >	30

In a first series of experiments, T-cell lines were generated from a healthy female individual (CT-10) with a history of genital exposure to *C. trachomatis* by stimulating T-cells with *C. trachomatis* LGV II elementary bodies as previously described. Although the study subject was exposed to *C. trachomatis*, she did not seroconvert and did not develop clinical symptoms, suggesting donor CT-10 may have developed a protective immune response against *C. trachomatis*. As shown in Fig. 10, a primary *Chlamydia*-specific T-cell line derived from donor CT-10 responded to *C. trachomatis*-SWIB, but not *C. pneumoniae*-SWIB recombinant proteins, confirming the exposure of CT-10 to *C. trachomatis*. Epitope mapping of the T-cell response to *C. trachomatis*-SWIB showed that this donor responded to the same epitope Ct-SWIB 52-67 (SEQ ID NO: 39) as T-cell line TCL-8, as shown in Fig. 11.

Additional T-cell lines were generated as described above for various C. trachomatis patients. A summary of the patients' clinical profile and proliferative responses to various C. trachomatis and C. pneumoniae elementary bodies and recombinant proteins are summarized in Table II.

Proliferative response of C. trachomatis patients										
atients	Clinical manifestation	IgG titer			CT Swib	- CP Swib	CT S13	CP S13	CT lpdA	CT TSA
CT-1	NGU	negative	+	+	-	-	++	++	++	+
CT-2	NGU	negative	++	++	-	-	+	+/-	-	-
СТ-3	asymptomatic shed Eb Dx was HPV	Ct 1:512 Cp 1:1024 Cps 1:256	+	+	-	-	+	-	+	-
CT-4	asymptomatic shed Eb	Ct 1:1024	+	+	-	-	-	- ·	-	-
CT-5	BV	Ct 1:256 Cp 1:256	++	++	-	-	+	-		-
СТ-6	perinial rash discharge	Cp 1:1024	+	+	-	-	-	-	-	-
CT-7	BV genital ulcer	Ct 1:512 Cp 1:1024	+	+	-	-	+	+	+	-
CT-8	Not known	Not tested	++	++	-	_	-	-	-	-
CT-9	asymptomatic	Ct 1:128 Cp 1:128	+++	++	-	-	++	+	+	-
CT-10	Itch mild vulvar	negative	++	++	-	-	-	-	-	-
CT-11	BV, abnormal pap	Ct 1: 512	+++	+++	-	-	+++-	+/-	++	+
CT-12	asymptomatic	Cp 1: 512	++	++	-	-	++	+	+	

NGU= Non-Gonococcal Urethritis; BV= Bacterial Vaginosis; CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant Chlamydia TSA protein
Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10⁵ PBMC with 1 x 10⁴ monocyte-derived dendritic cells pre-

incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a ³H-thymidine pulse for the last 18 hours.

DI. DI	muiation	nucx
+/-:	SI ~	4
+:	SI >	4
++:	SI	10-30

30

SI >

+++:

Using the panel of asymptomatic (as defined above) study subjects and *C. trachomatis* patients, as summarized in Tables I and II, a comprehensive study of the immune responses of PBMC derived from the two groups was conducted. Briefly, PBMCs from *C. pneumoniae* patients as well as from normal donors are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μg/ml gentamicin. Purified polypeptides, a panel of recombinant *chlamydial* antigens including *C. trachomatis-, C. pneumoniae-*SWIB and S13, as well as . *C. trachomatis* lpdA and TSA are added in duplicate at concentrations of 0.5 to 10 μg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μl, 50 μl of medium is removed from each well for determination of IFN-γ levels, as described below. The plates are then pulsed with 1 μCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

Proliferative responses to the recombinant Chlamydiae antigens demonstrated that the majority of asymptomatic donors and C. trachomatis patients recognized the C. trachomatis S13 antigen (8/12) and a majority of the C. trachomatis patients recognized the C. pneumonia S13 antigen (8/12), with 4/12 asymptomatic donors also recognizing the C. pneumonia S13 antigen. Also, six out of twelve of the C. trachomatis patients and four out of twelve of the asymptomatic donors gave a proliferative response to the lpdA antigen of C. trachomatis. These results demonstrate that the C. trachomatis and C. pneumonia S13 antigen, C. trachomatis Swib antigen and the C. trachomatis lpdA antigen are recognized by the asymptomatic donors, indicating these antigens were recognized during exposure to Chlamydia and an immune response elicited against them. This implies these antigens may

play a role in conferring protective immunity in a human host. In addition, the *C. trachomatis* and *C. pneumonia* S13 antigen is recognized equally well among the *C. trachomatis* patients, therefore indicating there may be epitopes shared between *C. trachomatis* and *C. pneumonia* in the S13 protein. Table III summarizes the results of these studies.

Table III.

Antigen	Normal Donors	C.t. Patients
C.tSwib	3/12	0/12
C.pSwib	0/12	0/12
C.tS13	8/12	8/12
C.pS13	4/12	8/12
lpdA	4/12	6/12
TSA	0/12	2/12

A series of studies were initiated to determine the cellular immune response to short-term T-cell lines generated from .asymptomatic donors and *C. trachomatis* patients. Cellular immune responses were measured by standard proliferation assays and IFN-γ, as described in Example 7. Specifically, the majority of the antigens were in the form of single *E. coli* clones expressing Chlamydial antigens, although some recombinant proteins were also used in the assays. The single *E. coli* clones were titered on 1 x 10⁴ monocyte-derived dendritic cells and after two hours, the culture was washed and 2.5 x 10⁴ T-cells were added. The assay using the recombinant proteins were performed as previously described. Proliferation was determined after four days with a standard ³H-thymidine pulse for the last 18 hours. Induction of IFN-γ was determined from culture supernatants harvested after four days using standard ELISA assays, as described above. The results show that all the *C. trachomatis* antigens tested, except for C.T. Swib, elicited a proliferative response from one or more different T-cell lines derived form *C. trachomatis* patients. In addition, proliferative responses were elicited from both the *C. trachomatis* patients and asymptomatic donors for

the following Chlamydia genes, CT622, groEL, pmpD, CT610 and rS13.

The 12G3-83-clone also contains sequences to CT734 and CT764 in addition to CT622, and therefore these gene sequence may also have immunoreactive epitopes. Similarly, clone 21G12-60 contains sequences to the hypothetical protein genes CT229 and CT228 in addition to CT875; and 15H2-76 also contains sequences from CT812 and CT088, as well as sharing homology to the sycE gene. Clone 11H3-61 also contains sequences sharing homology to the PGP6-D virulence protein.

Table IV.

Clone	C. t. Antigen	TCL from	TCL from	SEQ ID NO::
	(putative*)	Asymp. Donors	C. t. Patients	
1B1-66 (E. coli)	Swib	2/2	0/4	5
1B1-66 (protein)	Swib	2/2	0/4	5
12G3-83 (E. coli)	CT622*	2/2	4/4	57
22B3-53 (E. coli)	groEL	1/2	4/4	111
22B3-53 (protein)	groEL	1/2	4/4	111
15H2-76 (E. coli)	PmpD*	1/2	3/4	87
11H3-61 (E. coli)	rL1*	0/2	3/4	. 60
14H1-4 (E. coli)	TSA	0/2	3/4	56
14H1-4 (protein)	TSA	0/2	3/4	56
11G10-46 (E. coli)	CT610	1/2	1/4	62
10C10-17 (E. coli)	rS13	1/2	1/4	62
10C10-17 (protein)	rS13	1/2	1/4	62
21G12-60 (E. coli)	CT875*	0/2	2/4	110
11H4-32 (E. coli)	dnaK	0/2	2/4	59
21C7-8 (E. coli)	dnaK	0/2	2/4	115
17C10-31 (E. coli)	CT858	0/2	2/4	114

EXAMPLE 9 PROTECTION STUDIES USING CHLAMYDIA ANTIGENS

Protection studies were conducted in mice to determine whether immunization with chlamydial antigens can impact on the genital tract disease resulting from chlamydial inoculation. Two models were utilized; a model of intravaginal inoculation that uses a human isolate containing a strain of Chlamydia psittaci (MTW447), and a model of intrauterine inoculation that involves a human isolate identified as Chlamydia trachomatis, serovar F (strain NI1). Both strains induce inflammation in the upper genital tract, which resemble endometritis and salpingitis caused by Chlamydia trachomatis in women. In the first experiment, C3H mice (4 mice per group) were immunized three times with 100 µg of pcDNA-3 expression vector containing C. trachomatis SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5). Inoculations were at the base of the tail for systemic immunization. Two weeks after the last immunization, animals were progesterone treated and infected, either thru the vagina or by injection of the inoculum in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored (from + for very mild, to +++++ for very severe). Scores attributed to each single oviduct/ovary were summed and divided by the number of organs examined to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control-immunized animals receiving empty vector showed consistent inflammation with an ovary oviduct mean inflammation score of 6.12, in contrast to 2.62 for the DNA-immunized group. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 8.37, versus 5.00 for the DNA-immunized group. Also, in the later model, vaccinated mice showed no signs of tubal occlusion while negative control vaccinated groups had inflammatory cells in the lumen of the oviduct

In a second experiment, C3H mice (4 mice per group) were immunized three times with 50 µg of pcDNA-3 expression vector containing *C. trachomatis* SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5) encapsulated in Poly Lactide co-Glycolide microspheres (PLG); immunizations were made

intra-peritoneally. Two weeks after the last immunization, animal were progesterone treated and infected by inoculation of *C. psittaci* in the vagina. Two weeks after infection, mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored as previously described. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean of inflammation for the group. Negative control-immunized animals receiving PLG-encapsulated empty vector showed consistent infammation with an ovary /oviduct mean inflammation score of 7.28, versus 5.71 for the PLG-encapsulated DNA immunized group. Inflammation in the peritoneum was 1.75 for the vaccinated group versus 3. 75 for the control.

In a third experiment, C3H mice (4 per group) were immunized three times with 10 µg of purified recombinant protein, either SWIB (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5, or S13 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12) mixed with Cholera Toxin (CT); the preparation was administred intranasally upon anaesthesia in a 20 uL volume. Two weeks after the last immunization, animal were progesterone treated and infected, either by vaginal inoculation of C. psittaci or by injection of C. trachomatis serovar F in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. The degree of inflammation was scored as described above. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control- immunized animals receiving cholera toxin alone showed an ovary /oviduct mean inflammation score of 4.25 (only 2 mice analyzed; 2 other died) versus 5.00 for the s13 plus cholera toxin-immunized group, and 1.00 for the SWIB plus cholera toxin. Untreated infected animals had an ovary/oviduct mean inflammation score of 7. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 7.37 versus 6.75 for the s13 plus cholera toxin-immunized group and 5.37 for the SWIB plus cholera toxin-immunized group. Untreated infected animals had an ovary/oviduct mean inflammation score of 8.

The three experiments described above suggest that SWIB-specific protection is obtainable. This protective effect is more marked in the model of homologous infection but is still present when in a heterologous challenge infection with *C. psittaci*.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

Claims

- 1. An isolated polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290; (b) sequences complementary to a sequence of (a); and (c) polynucleotide sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 2. The polypeptide of claim 1 wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 5, 26, 32, 65, 90, 92-98, 103-108, 121, 123, 125, 127, 129, 131, 133, 135, 137, 175-180, 189-196, 264 and 266.
- 3. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1 and 2.
- 4. A recombinant expression vector comprising a polynucleotide molecule according to claim 3.
 - 5. A host cell transformed with an expression vector according to claim 4.
- 6. The host cell of claim 5 wherein the host cell is selected from the group consisting of *E. coli*, yeast and mammalian cells.
- 7. A fusion protein comprising a polypeptide according to any one of claims 1 and 2.
- 8. A fusion protein according to claim 7, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell

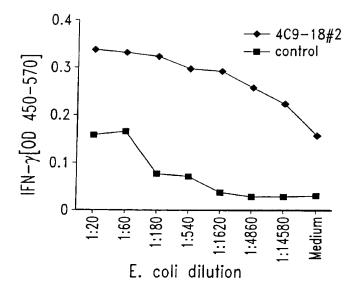


Fig. 1

2/10

Retroviral vector pBIB-KS - -KS-MCS IRES-Blastor LTR Kozak-Start GA TCT GCC GCC ACC ATG GAA TTC GAT ATC GGA TCC CTG CAG A CGG CGG TGG TAC CTT AAG CTA TAG CCT AGG GAC GTC (BglII) EcoRI BamHI ReadingFrame 1 AAG CTT GAG CTC GAG CGC GGC CGC TAA TITA GCT GAG KS1+ TTC GAA CTC GAG CTC GCG CCG GCG ATT AAT CGA CTC AGC T Stop Stop Stop (Sall) XhoI NotI Kozak-Start GA TCT GCC GCC ACC ATG GA ATT CGA TAT CGG ATC CCT GCA G <u>A</u> CGG CGG TGG TAC C<u>CT TAA G</u>CT ATA G<u>CC TAG GGA CGT C</u> (BglII) **EcoRI** BamHI ReadingFrame 1 AA GCT TGA GCT CGA GCG CGG CCG QTA ATIT AGC TGA G KS2+ TT CGA ACT CGA GCT CGC GCC GGC GAT THA TCG ACT CAG CT Stop Stop Stop (Sall) HinDIII XhoI NotI Kozak-Start GA TCT GCC GCC ACC ATG GGG AAT TCG ATA TCG GAT CCC TGC AG A CGG CGG TGG TAC CCC TTA AGC TAT AGC CTA GGG ACG TC **EcoRI** BamHI PstI ReadingFrame 3 A AGC TTG AGC TCG AGC GCG GCC GCT AAT TAG CTG AG KS3+ T TCG AAC TCG AGC TCG CGC CGG CGA TTA ATC GAC TCA GCT Stop Stop Stop (Sall) HinDIII XhoI NotI

Fig. 2

Chlamydia C17.8 Peptide Screen

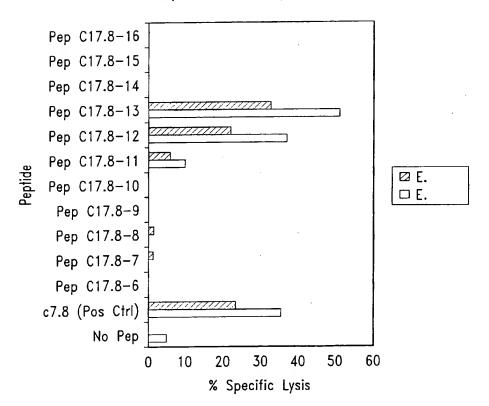
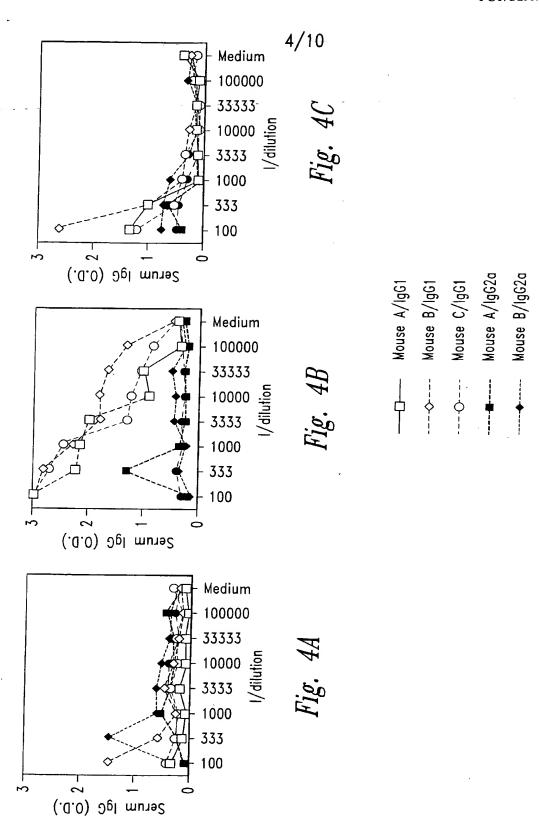
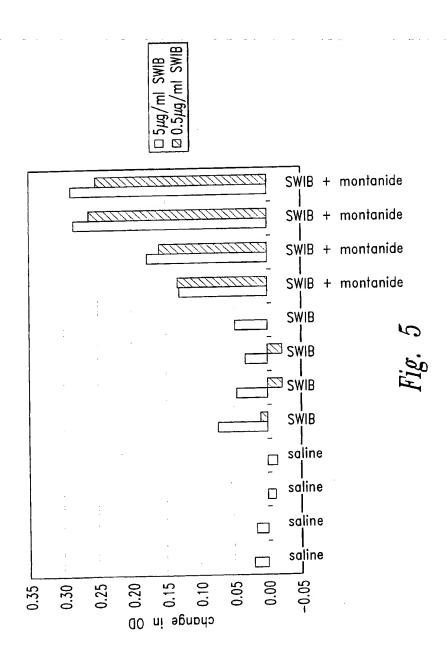


Fig. 3



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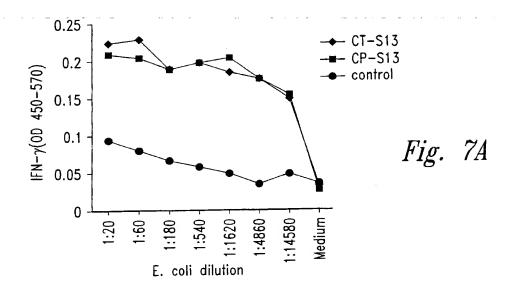
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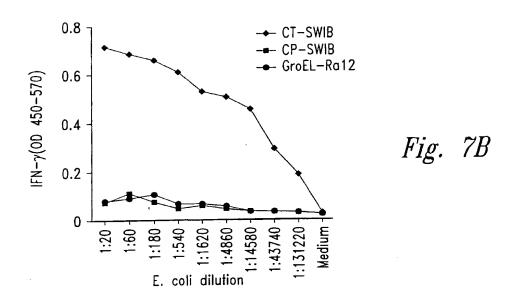
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CP S13 EcoRI (3' primer)
5' CTCGAGGAATTCTTATTTCTTCTTACCTGC

Fig. 6





skin teilteis immilisit kan oo oo kalles keisistilisisti teknii iimitti koo kallanta kan in kasii in kalii ista

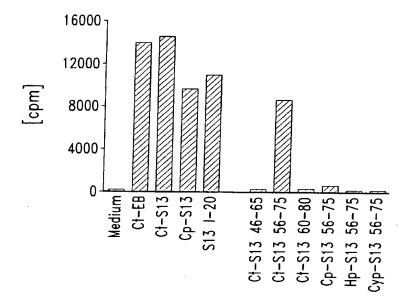
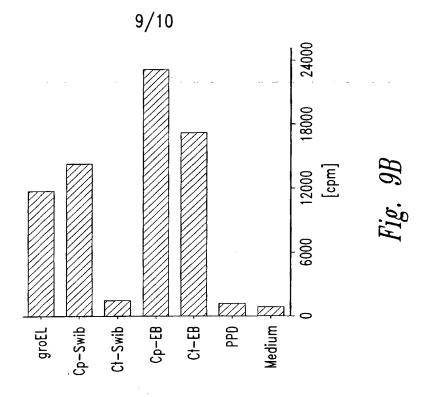
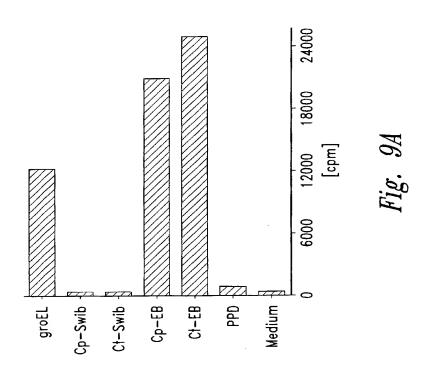
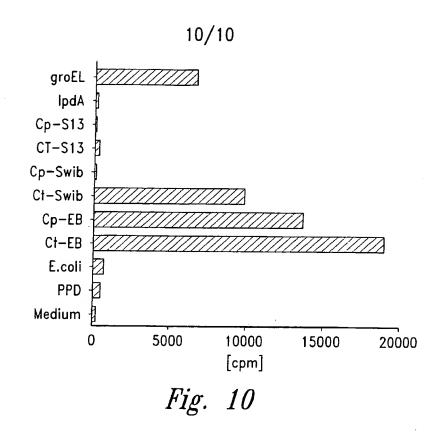
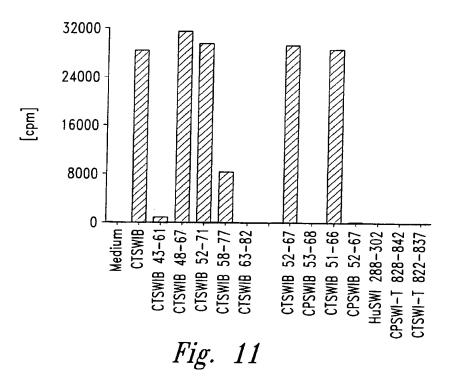


Fig. 8









SUBSTITUTE SHEET (RULE 26)

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SEQUENCE LISTING

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Trp Leu Leu Asp Val Arg Ser Leu Leu Gln Leu Leu Asp Cys Ala Leu
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adisantanakan an kanakan mengan m Tanggan mengan meng

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Gly	Ser	Glu 35	Val	Ser	Val	Ile	Glu 40	Ala	Ser	Ser	Gln	Ile 45	Leu	Ala	Leu
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Gly 65	Leu	Arg	Phe	Val	Leu 70	Glu	Ala	Ser	Val	Ser 75	Asn	Ile	Glu	Asp	Ile 80
Gly	Asp	Arg	Val	Arg 85	Leu	Thr	Ile	Asn	Gly 90	Asn	Val	Glu	Glu	Tyr 95	Asp
Tyr	Val	Leu	Val 100	Ser	Ile	Gly	Arg	Arg 105	Leu	Asn	Thr	Glu	Asn 110	Ile	Gly
Leu	Asp	Lys 115	Ala	Gly	Val	Ile	Cys 120	Asp	Glu	Arg	Gly	Val 125	Ile	Pro	Thr
Asp	Ala 130	Thr	Met	Arg	Thr	Asn 135	Val	Pro	Asn	Ile	Tyr 140	Ala	Ile	Gly	Asp
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Ile	Ala	Ala	Arg	Asn 165	Ile	Gly	Gly	His	Lys 170	Glu	Glu	Ile	Asp	Tyr 175	Ser
Ala	Val	Pro	Ser 180	Val	Ile	Phe	Thr	Phe 185	Pro	Glu	Val	Ala	Ser 190	Val	Gly
Leu	Ser	Pro 195	Thr	Ala	Ala	Gln	Gln 200	His	Leu	Leu	Leu	Arg 205	Leu	Leu	Phe
	Lys 210	Asn	Leu	Ile	Gln	Lys 215	Lys	Asn	Ser		His 220	Thr	Cys	Glu	Glυ
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ક્ષાના ભાગામાં આવેલા કાર્યા કરવા માન્યા માત્ર કરવા માત્ર કરવા માત્ર માત્ર માત્ર કરવા માત્ર કરવા માત્ર કરવા માત આ માત્ર માત્ર માત્ર માત્ર માત્ર માત્ર માત્ર માત્ર માત્ર કરવા માત્ર માત્ર માત્ર માત્ર માત્ર માત્ર માત્ર માત્ર મ

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Thr Glu Glu Glu Val Gly Arg Leu Asn Ser Leu Leu Gln Ser Glu Tyr
Thr Val Glu Gly Asp Leu Arg Arg Val Gln Ser Asp Ile Lys Arg
                                        75
Leu Ile Ala Ile His Ser Tyr Arg Gly Gln Arg His Arg Leu Ser Leu
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                                 25
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gacaaaaata caaaggaggt tcactcctaa ccagaaaaag ggagagttag tttccatqqq 240
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TERMERSET PROGREGEREN FRED FRED FRED FOR FRESKRIKKEN HILLING FOR FOLKSKIKE FOR FRESKRIKEN FRESKRIKEN.

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PCT/US99/29012

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ANTAGERIA DE LA CONTRACTORIA DE LA

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aldiktlistlisatsistillistlatist killistlatist kalitain indan organista organista killistista killistista kalitai killista kalitai kali

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      <211> 399
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                                                                   39.9
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<213> Chlamydia

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Phe Met His Pro Val Asn Ile Ser Thr Asp Leu Ala Val Ile Val Gly
20 25 30

Lys Gly Pro Met Pro Arg Thr Glu Ile Val Lys Lys Val Trp Glu Tyr

Ile Lys Lys His Asn Cys Gln Asp Gln Lys Asn Lys Arg Asn Ile Leu
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Gly Thr Cys Leu Asn Arg Gly Cys Ile Pro Ser Lys Ala Leu Leu Ala
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Gly Ala Glu Val Val Thr Gln Ile Arg His Ala Asp Gln Phe Gly Ile
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His Val Glu Gly Phe Ser Ile Asn Tyr Pro Ala Met Val Gln Arg Lys 85 90 95

Asp Ser Val Val Arg Ser Ile Arg Asp Gly Leu Asn Gly Leu Ile Arg

Ser Asn Lys Ile Thr Val Phe Ser Gly Arg Gly Ser Leu Ile Ser Ser 115 120 125

Thr Glu Val Lys Ile Leu Gly Glu Asn Pro Ser Val Ile Lys Ala His 130 135 140

Ser Ile Ile Leu Ala Thr Gly Ser Glu Pro Arg Ala Phe Pro Gly Ile 145 150 155 160

nealthaanatta tana tabaatan sa sa hasa tama makaman makaman makama ay ing ing ing ing ing makhada kathada ta i

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- Asn Leu Lys Glu Ile Pro Gln Lys Met Ala Ile Ile Gly Gly Gly Val 180 185 190
- Ile Gly Cys Glu Phe Ala Ser Leu Phe His Thr Leu Gly Ser Glu Val
- Ser Val Ile Glu Ala Ser Ser Gln Ile Leu Ala Leu Asn Asn Pro Asp 210 215 220
- Ile Ser Lys Thr Met Phe Asp Lys Phe Thr Arg Gln Gly Leu Arg Phe 225 230 240
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- Arg Leu Thr Ile Asn Gly Asn Val Glu Glu Tyr Asp Tyr Val Leu Val
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- Ser Ile Gly Arg Arg Leu Asn Thr Glu Asn Ile Gly Leu Asp Lys Ala 275 280 285
- Gly Val Ile Cys Asp Glu Arg Gly Val Ile Pro Thr Asp Ala Thr Met 290 295 300
- Arg Thr Asn Val Pro Asn Ile Tyr Ala Ile Gly Asp Ile Thr Gly Lys 305 310 315 320
- Trp Gln Leu Ala His Val Ala Ser His Gln Gly Ile Ile Ala Ala Arg 325 330 335
- Asn Ile Gly Gly His Lys Glu Glu Ile Asp Tyr Ser Ala Val Pro Ser 340 345 350
- Val Ile Phe Thr Phe Pro Glu Val Ala Ser Val Gly Leu Ser Pro Thr 355 360 365
- Ala Ala Gln Gln Lys Ile Pro Val Lys Val Thr Lys Phe Pro Phe 370 380
- Arg Ala Ile Gly Lys Ala Val Ala Met Gly Glu Ala Asp Gly Phe Ala 385 390 395 400
- Ala Ile Ile Ser His Glu Thr Thr Gln Gln Ile Leu Gly Ala Tyr Val 405 410 415
- Ile Gly Pro His Ala Ser Ser Leu Ile Ser Glu Ile Thr Leu Ala Val
 420 425 430
- Arg Asn Glu Leu Thr Leu Pro Cys Ile Tyr Glu Thr Ile His Ala His 435 440 445
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Glu Ala Arg Ala Ser Glu Leu Thr Glu Glu Glu Val Gly Arg Leu Asn 50 55 60

Ser Leu Leu Gln Ser Glu Tyr Thr Val Glu Gly Asp Leu Arg Arg Arg 65 70 75 80

Val Gln Ser Asp Ile Lys Arg Leu Ile Ala Ile His Ser Tyr Arg Gly 85 90 95

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Lys

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Lys Asp Phe Thr Tyr Val Cys Pro Thr Glu Leu His Ala Phe Gln Asp

Arg Leu Val Asp Phe Glu Glu His Gly Ala Val Leu Gly Cys Ser

Val Asp Asp Ile Glu Thr His Ser Arg Trp Leu Thr Val Ala Arg Asp 90

Ala Gly Gly Ile Glu Gly Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser

Phe Lys Ile Ser Glu Ala Phe Gly Val Leu Asn Pro Glu Gly Ser Leu 120

Ala Leu Arg Ala Thr Phe Leu Ile Asp Lys His Gly Val Ile Arg His 135

Ala Val Ile Asn Asp Leu Pro Leu Gly Arg Ser Ile Asp Glu Glu Leu 150 155

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lytheen folkulusteen measte and the film of the control of the con

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780-

840

897

<213> Chlamydia

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65		_		_	70	_		-1	_	75		_	_	~ 3	8 0	
Thr	vaı	ьeu	Ala	ьеи 85	GIY	Asn	Ата	Pne	Asn 90	GIY	Ата	Leu	Pro	95	Thr	
Val	Gln	Ser	Ala		Ser	Phe	Phe	Ser		Met	Lys	Ala	Ala		Gln	
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Arq	Cys		Arg	Ile	Ala	Arq		Glu	Ser	Ser	Leu		Leu	Ser	Glv	
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	Glu	Asn	Ala	CAa		Arg	Arg	Val	Ala		Glu	Lys	Ala	Lys		
225	Mlb	7	- 1-	T	230	77-	T	T	ml	235	T	a 1	T	To 1	240	
Pne	int	Arg	Ile	ьуs 245	ıyr	Ата	ьeu	ьeu	250	мет	Leu	GIU	ьys	255	Leu	
Glu	Cys	Val	Ala		Val	Phe	Lys	Leu		Pro	Leu	Pro	Ile		Met	
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Ile	Gly 290	Leu	Trp	Thr	Phe	Cys 295	Ala	Arg	Ala							
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                                105
Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser
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His Lys Arg Arg Ala Ala Ala Val Cys Gly Phe Ile Gly Gly Ile
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Thr Tyr Leu Ala Thr Phe Gly Val Ile Arg Pro Ile Leu Phe Val Asn
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Lys Met Leu Val Asn Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
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Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
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Val Gly Ala Gly Leu Ala Ile Ser Ala Glu Arg Ala Asp Cys Glu Ala
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                            200
                                                205
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Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
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Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg
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                                        75
Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr
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                                    90
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Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn

135

105

110

125

140

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Lys Thr Lys Gly Met Asp Lys Thr Ile Lys Val Ala Lys Ser Ala Ala
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Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
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Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser
                            120
His Lys Arg Arg Ala Ala Ala Val Cys Ser Ile Ile Gly Gly Ile
                        135
Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
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Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
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Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
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                                185
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                            200
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                                        235
Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
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della elektrida ekikaristaria kalitalikakitapaka in bagir mamman papa kula katatah pilara akitalitai ilikakithi melikataki ambi

hala shi ne ni sa is sa kata kini ma rain ir

PCT/US99/29012

60

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acacagecca geaataaaat ggeaagggta gtaaataaga egaagggaat ggataagaet 120 gttaaggteg ecaagtetge tgeegaattg acegeaaata ttttggaaca agetggagge 180 gegggetett egeacacat tacagettee eaagtgteea aaggattagg ggatgegaga 240 actgtteetg etttagggaa tgeetttaac ggagegttge eaggaacagt teaaagtgeg 300

420

480

540

600

660

720

780

840

897

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Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
                        55
Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arq
Thr Val Leu Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Prc Gly Thr
                                    90
Val Gln Ser Ala Gln Ser Phe Phe Ser Tyr Met Lys Ala Ala Ser Gln
                                105
Lys Pro Gln Glu Gly Asp Glu Gly Leu Val Ala Asp Leu Cys Val Ser
                         . 120
His Lys Arg Arg Ala Ala Ala Val Cys Ser Phe Ile Gly Gly Ile
                        135
                                            140
Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
                    150
                                        155
Lys Met Leu Ala Gln Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
                                    170
                165
Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Phe Val
                                185
                                                    190
Val Gly Ser Gly Leu Ala Ile Ser Ala Glu Arg Ala Asp Cys Glu Ala
                            200
Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Ser Leu Glu Leu Ser Gly
                        215
Glu Glu Asn Ala Cys Glu Arg Gly Val Ala Gly Glu Lys Ala Lys Thr
                    230
                                        235
Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
                245
                                    250
Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
            260
                               265
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Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Val
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                                               285
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HarlissUhssan) hiddinamainistika orahin katoo orang magada gaga percenging Anghilipi Harlisi kalikatishkin tahunkin katoo ola bakka disahni

Ile Gly Leu Trp Thr Phe Cys Asn Arg Val

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180

300

420

480

540

600

660

720

780

840

897

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gttaaggtcg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc
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caaagettet tetettacat gaaagetget agteagaaac egeaagaagg ggatgagggg
ctestageag atctttgtgt gteteataag egeagagegg etgeggetgt etgtagette
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gaattgtcgg gagaggaaaa tgcttgtgag aggagagtcg ctggagagaa agccaagacg
ttcacgcgca tcaagtatgc actcctcact atgctcgaga agtttttgga atgcgttgcc
gacgtttttca aattggtgcc gttgcctatt acaatgggta ttcgtgcaat tgtggctqcq
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        35
                            40
Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
                        55
                                            60
Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg
                    70
                                        75
Thr Val Leu Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr
                85
                                    90
                                                        95
Val Gln Ser Ala Gln Ser Phe Phe Ser Tyr Met Lys Ala Ala Ser Gln
                                105
                                                    110
Lys Pro Gln Glu Gly Asp Glu Gly Leu Val Ala Asp Leu Cys Val Ser
                            120
                                                125
His Lys Arg Arg Ala Ala Ala Val Cys Ser Phe Ile Gly Gly Ile
                        135
                                            140
Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
                                        155
Lys Met Leu Ala Gln Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
                                                        175
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Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Phe Val

Val Gly Ser Gly Leu Ala Ile Ser Ala Glu Arg Ala Asp Cys Glu Ala

200

185

190

180

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Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Ser Leu Glu Leu Ser Gly
                         215
Glu Glu Asn-Ala Cys Glu Arg Arg Val Ala Gly Glu Lys Ala Lys Thr
225
                     230
                                         235
Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
                 245
                                     250
Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
                                 265
Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Val
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Ile Gly Leu Trp Thr Phe Cys Asn Arg Val
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      <211> 897
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                                                                       120
attaaggttg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc
gcgggctctt ccgcacacat tacagcttcc caagtgtcca aaggattagg ggatgcgaga
                                                                        240
actifttigtcg ctttagggaa tgcctttaac ggagcgttigc caggaacagt tcaaagtigcg
                                                                        300
caaagcttct tctctcacat gaaagctgct agtcagaaaa cgcaagaagg ggatgagggg
                                                                       360
ctcacagcag atctttgtgt gtctcataag cgcagagcgg ctgcggctgt ctgtagcatc
                                                                       420
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                                                                       480
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                                                                       540
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                                                                       600
gcggaaagag cagattgcga agcccgctgc gctcgtattg cgagagaaga gtcgttactc
                                                                       660
gaaatgccgg gagaggaaaa tgcttgcgag aagaaagtcg ctggagagaa agccaagacg
                                                                       720
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                                                                       780
gacgttttca aattggtgcc gctgcctatt acaatgggta ttcqtqcqat tqtqqctqct
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                                25
Lys Thr Lys Gly Met Asp Lys Thr Ile Lys Val Ala Lys Ser Ala Ala
                            40
Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg
                    70
                                        75
```

Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr

Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln

105

ૠ૽૽ૡૺઌૺઌઌઌ૽ૢઌ૽ઌ૽ઌઌ૽ઌ૽ૡ૽ૡ૽૽ૡ૽ૹ૽૱ૹૢૡઌૢઌ૽ૢ૽ૢૡ૽ૡઌ૽ઌ૽૽ઌઌઌઌઌઌઌઌઌઌઌ૽ૺઌઌ૽ૹ૽ઌઌ૽ૹ૽૽ઌઌ૽ૺઌઌ૽૽ઌ૿૽ઌ૿૽ઌ૽૽ઌ૽૽ઌ૽૽ઌ૿૽ઌ૽૽ઌ૽૽ઌ૽૽ઌ૽ૺ

90 .

85

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                         135
 Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
                     150
                                         155
Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
                 165
                                     170
Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
                                 185
Val Gly Ala Gly Leu Ala Ile Ser Ala Glu Arg Ala Asp Cys Glu Ala
                             200
Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Met Pro Gly
                                             220
Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr
                    230
                                         235
Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
                245
                                     250
Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
            260
                                 265
Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile
                            280
                                                 285
Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala
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                                                                       120
ataaaggttg ggaagtctgc tgctgaatta acggcgagta ttttagagca aactgggggg
                                                                       180
gcagggactg atgcacatgt tacggcggcc aaggtgtcta aagcacttgg ggacgcgcga
                                                                       240
acagtaatgg ctctagggaa tgtcttcaat gggtctgtgc cagcaaccat tcaaagtgcg
                                                                       300
cgaagctgtc tcgcccattt acgagcggcc ggcaaagaag aagaaacatg ctccaaggtg
                                                                       360
aaagatetet gtgtttetea tagaegaaga getgeggetg aggettgtaa tqttattqqa
                                                                       420
ggagcaactt atattacaac tttcggagcg attcgtccga cattactcgt taacaaqctt
                                                                       480
cttgccaaac cattcctttc ctcccaagcc aaagaagggt tgggagcttc tgttggttat
                                                                       540
atcatggcag cgaaccatgc ggcatctgtg cttgggtctg ctttaagtat tagcgcagaa
                                                                       600
agagcagact gtgaagagcg gtgtgatcgc attcgatgta gtgaggatgg tgaaatttgc
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gaaggcaata aattaacagc tatttcggaa gagaaggcta gatcatggac tctcattaag
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tacagattcc ttactatgat agaaaaacta tttgagatgg tggcggatat cttcaagtta
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attectitge caattiegea tggaattegt getattgitg etgegggatg taegttgaet
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      <212> PRT
      <213> Chlamydia
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<220>

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Glu Leu Thr Ala Ser Ile Leu Glu Gln Thr Gly Gly Ala Gly Thr Asp
                        55
Ala His Val Thr Ala Ala Lys Val Ser Lys Ala Leu Gly Asp Ala Arg
                    70
                                       75
Thr Val Met Ala Leu Gly Asn Val Phe Asn Gly Ser Val Pro Ala Thr
                                   90
Ile Gln Ser Ala Arg Ser Cys Leu Ala His Leu Arg Ala Ala Gly Lys
                               105
Glu Glu Glu Thr Cys Ser Lys Val Lys Asp Leu Cys Val Ser His Arg
                           120
Arg Arg Ala Ala Ala Glu Ala Cys Asn Val Ile Gly Gly Ala Thr Tyr
                       135
Ile Thr Thr Phe Gly Ala Ile Arg Pro Thr Leu Leu Val Asn Lys Leu
                  150
                                      155
Leu Ala Lys Pro Phe Leu Ser Ser Gln Ala Lys Glu Gly Leu Gly Ala
               165
                                   170
Ser Val Gly Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val Leu Gly
                               185
           180
                                                   190
Ser Ala Leu Ser 11e Ser Ala Glu Arg Ala Asp Cys Glu Glu Arg Cys
                           200
Asp Arg Ile Arg Cys Ser Glu Asp Gly Glu Ile Cys Glu Gly Asn Lys
                       215
                                           220
Leu Thr Ala Ile Ser Glu Glu Lys Ala Arg Ser Trp Thr Leu Ile Lys.
                   230
                                       235
Tyr Arg Phe Leu Tnr Met Ile Glu Lys Leu Phe Glu Met Val Ala Asp
                245
                                   250
Ile Phe Lys Leu Ile Pro Leu Pro Ile Ser His Gly Ile Arg Ala Ile
                       265
          260
Val Ala Ala Gly Cys Thr Leu Thr Ser Ala Val Ile Gly Leu Gly Thr
                           280
       275
Phe Trp Ser Arg Ala
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     <212> PRT
     <213> Artificial Sequence
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and demonstrational translation of the properties of the properties of the properties of the properties of the

DIBURGERENTES LINGVERSE SKELLEG FOR EN EKKELIKUNG KERTES KAKET PROM

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Arg Ala Ala Ala Val Cys Ser Phe Ile Gly Gly Ile Thr Tyr Leu
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Ser Gln
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     <223> Made in a lab
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Ser
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     <223> Made in a lab
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     <210> 146
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     <400> 146
Phe Ile Gly Gly Ile Thr Tyr Leu
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     <211> 9
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Cys Ser Phe Ile Gly Gly Ile Thr
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      <211> 10
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      <223> Made in a lab
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Cys Ser Ile Ile Gly Gly Ile Thr Tyr Leu
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                                    10
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      <211> 9
      <212> PRT
      <213> Artificial Sequence
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Gly Phe Ile Gly Gly Ile Thr Tyr Leu
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Ser Val Ala Ser
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Thr Ser Arg His
          20
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     <211> 20
     <212> PRT
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Arg Phe Cys Leu
          20
     <210> 155
      <211> 20
     <212> PRT
     <213> Artificial Sequence
     <220>
     <223> Made in a lab
     <400> 155
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Arg Asn Arg Phe
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      <211> 20
      <212> PRT
      <213> Artificial Sequence
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<211> 24 <212> DNA

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Gln Ile Trp Asp
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      <210> 157
      <211> 53
      <212> PRT
      <213> Artificial Sequence
      <220>
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      <400> 157
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3
                                    10
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                                25
Phe Cys Leu Ser Thr Lys Cys Trp Arg Asn Arg Phe Phe Leu Pro Lys
                           40
Leu Lys Gln Ile Trp
    50
      <210> 158
      <211> 52
      <212> PRT
      <213> Artificial Sequence
      <220>
      <223> Made in a lab
      <400> 158
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                                    10
Ile Gly Gly Ile Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile
                                25
                                                    30
Leu Phe Val Asn Lys Met Leu Ala Gln Pro Phe Leu Ser Ser Gln Ile
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Lys Ala Asn Met
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      <211> 24
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      <210> 160
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Glu Asp Cys Thr Met Glu Ser Leu Phe Pro Ala Leu Cys Ala His Ala
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			Trp	565					570					575	
Ser	TIII	ьeu	Val	нта	ASΠ	TIII.	ьеи	тrр	ASII	T11 T ,	TAL	ser	ASP	MAC	GTII

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580
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 Ala Val Gln Ser Met Ile Asn Thr Thr Ala His Gly Gly Ala Tyr Leu
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 Phe Gly Thr Trp Gly Ser Ala Val Ser Asn Leu Phe Tyr Val His Asp
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 Ser Ser Gly Lys Pro Ile Asp Asn Trp His His Arg Ser Leu Gly Tyr
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                                      635
 Leu Phe Gly Ile Ser Thr His Ser Leu Asp Asp His Ser Phe Cys Leu
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Ala Ala Gly Gln Leu Leu Gly Lys Ser Ser Asp Ser Phe Ile Thr Ser
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                              665
Thr Glu Thr Thr Ser Tyr Ile Ala Thr Val Gln Ala Gln Leu Ala Thr
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                           680
                                               685
Ser Leu Met Lys Ile Ser Ala Gln Ala Cys Tyr Asn Glu Ser Ile His
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Glu Leu Lys Thr Lys Tyr Arg Ser Phe Ser Lys Glu Cly Phe Gly Ser
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                                       715
Trp His Ser Val Ala Val Ser Gly Glu Val Cys Ala Ser Ile Pro Ile
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                                   730
Val Ser Asn Gly Ser Gly Leu Phe Ser Ser Phe Ser Ile Phe Ser Lys
                               745
Leu Gln Gly Phe Ser Gly Thr Gln Asp Gly Phe Glu Glu Ser Ser Gly
                           760
                                              765
Glu Ile Arg Ser Phe Ser Ala Ser Ser Phe Arg Asn Ile Ser Leu Pro
                      775
                                          780
Ile Gly Ile Thr Phe Glu Lys Lys Ser Gln Lys Thr Arg Thr Tyr Tyr
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                                      795
Tyr Phe Leu Gly Ala Tyr Ile Gln Asp Leu Lys Arg Asp Val Glu Ser
               805
                                   810
Gly Pro Val Val Leu Leu Lys Asn Ala Val Ser Trp Asp Ala Pro Met
                              825 .
Ala Asn Leu Asp Ser Arg Ala Tyr Met Phe Arg Leu Thr Asn Gln Arg
                           840
                                              845
Ala Leu His Arg Leu Gln Thr Leu Leu Asn Val Ser Cys Val Leu Arg
                       855
                                        860
Gly Gln Ser His Ser Tyr Ser Leu Asp Leu Gly Thr Thr Tyr Arg Phe
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Pro Tyr Thr Val Ile Gly Asp Pro Ser Gly Thr Thr Val Phe Ser Ala
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                              25
Gly Glu Leu Thr Leu Lys Asn Leu Asp Asn Ser Ile Ala Ala Leu Pro
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	Leu	Ser 50	Cys	Phe	Gly	Asn	Leu 55	Leu	Gly	Ser	Phe	Thr 60	Val	Leu	Gly	Arg
-	Gly 65		Ser	Leu	Thr	Phe 70	Glu	Asn	Ile	Arg	Thr 75	Ser	Thr	Asn	Gly	Ala 80
	Ala	Leu	Ser	Asn	Ser 85	Ala	Ala	Asp	Gly	Leu 90	Phe	Thr	Ile	Glu	Gly 95	Phe
	Lys	Glu	Leu	Ser 100	Phe	Ser	Asn	Cys	Asn 105	Ser	Leu	Leu	Ala	Val 110	Leu	Pro
	Ala	Ala	Thr 1.15	Thr	Asn	Lys	Gly	Ser 120	Gln	Thr	Pro	Thr	Thr 125	Thr	Ser	Thr
		130					135					140			Leu	
	145					150					155				Gly	160
					165					170					Leu 175	
				180		-			185					190	Gln	
			195					200					205		Phe	
		210					215					220			Gln	
	225					230					235				Val	240
					245					250					Ala 255	
				260					265					270	Asn	
			275					280					285		Ile	
		290					295					300			Asn	
	305	_	_			310					315				Gly	320
					325					330					Phe 335 Lys	
				340					345					350	Ile	
			355					360					365		Ser	
		370					375					380			Arg	
	385					390					395				Ser	400
					405					410					415 Ala	
				420					425					430	Asn	
			435	•				440					445		Asp	
		450					455					460			Thr	
	465	_				470					475				Glu	480
	ı yr	GIN	ASII	vdl	1111	тте	GIU	GTII	GIA	Ar 9	116	vaı	пси	- 1 − 1	JIU	-y 3

				485					490					49	5
			500)				505	;				51	0	ı Tyr
		515	5				520					529	5		o Gln
	530)				535					540)			s Leu
Ser 545		Ser	Ser	Leu	Leu 550		Asn	Asn	Ala	Va]		Asr	ı Pro	Pro	Thr 560
				565					570)		_		575	
			580					585					590)	a Asp
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	610					615					620				Pro
625					630					635					Gly 640
				645					650					655	Tyr
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	690				Ser	695					700				
705					Trp 710					715					720
				725	Gly				730					735	
			740		Ser			745					750		
		755			Gly		760					765			
	770				Ile	775					780				
785					Leu 790					795					800
				805	His				810					815	
			820		Asp			825					830	_	
		835			Ile		840					845			
	850					855					860				
865					Gln 870					875					Leu 880
				885	Val				890					895	Thr
			900		Ser			905					910		
Arg	Thr	Ile 915	Ser	Gly	Thr		Thr ' 920	Thr	Leu	Leu	Ser	His 925	Gln	Glu	Thr

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Trp Thr Thr Asp Ala Phe His Leu Ala Arg His Gly Val Val Val Arg
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Gly Ser Met Tyr Ala Ser Leu Thr Ser Asn Ile Glu_Val Tyr Gly His
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Gly Arg Tyr Glu Tyr Arg Asp Ala Ser Arg Gly Tyr Gly Leu Ser Ala
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Gly Ser Lys Val Xaa Phe
            980
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Pro Asp Pro Thr Lys Glu Ser Leu Ser Asn Lys Ile Ser Leu Thr Gly
Asp Thr His Asn Leu Thr Asn Cys Tyr Leu Asp Asn Leu Arg Tyr Ile
                       55
Leu Ala Ile Leu Gln Lys Thr Pro Asn Glu Gly Ala Ala Val Thr Ile
                   70
                                       75
Thr Asp Tyr Leu Ser Phe Phe Asp Thr Gln Lys Glu Gly Ile Tyr Phe
                                   90
              85
Ala Lys Asn Leu Thr Pro Glu Ser Gly Gly Ala Ile Gly Tyr Ala Ser
          100
                               105
Pro Asn Ser Pro Thr Val Glu Ile Arg Asp Thr Ile Gly Pro Val Ile
                           120
Phe Glu Asn Asn Thr Cys Cys Arg Leu Phe Thr Trp Arg Asn Pro Tyr
                       135
Ala Ala Asp Lys Ile Arg Glu Gly Gly Ala Ile His Ala Gln Asn Leu
                                      155
                   150
Tyr Ile Asn His Asn His Asp Val Val Gly Phe Met Lys Asn Phe Ser
               165
                                   170
Tyr Val Gln Gly Gly Ala Ile Ser Thr Ala Asn Thr Phe Val Val Ser
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           180
Glu Asn Gln Ser Cys Phe Leu Phe Met Asp Asn Ile Cys Ile Gln Thr
                          200
Asn Thr Ala Gly Lys Gly Gly Ala Ile Tyr Ala Gly Thr Ser Asn Ser
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                      215
Phe Glu Ser Asn Asn Cys Asp Leu Phe Phe Ile Asn Asn Ala Cys Cys
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                                      235
Ala Gly Gly Ala Ile Phe Ser Pro Ile Cys Ser Leu Thr Gly Asn Arg
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               245
Gly Asn Ile Val Phe Tyr Asn Asn Arg Cys Phe Lys Asn Val Glu Thr
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Ala Ser Ser Glu Ala Ser Asp Gly Gly Ala Ile Lys Val Thr Thr Arg
                          280
                                             285
Leu Asp Val Thr Gly Asn Arg Gly Arg Ile Phe Phe Ser Asp Asn Ile
                                         300
                    295
Thr Lys Asn Tyr Gly Gly Ala Ile Tyr Ala Pro Val Val Thr Leu Val
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315

One with the word of the control of

Asp Asn Gly Pro Thr Tyr Phe Ile Asn Asn Ile Ala Asn Asn Lys Gly 325 Gly Ala Ile Tyr Ile Asp Gly Thr Ser Asn Ser Lys Ile Ser Ala Asp 345 Arg His Ala Ile Ile Phe Asn Glu Asn Ile Val Thr Asn Val Thr Asn 360 Ala Asn Gly Thr Ser Thr Ser Ala Asn Pro Pro Arg Arg Asn Ala Ile 375 380 Thr Val Ala Ser Ser Ser Gly Glu Ile Leu Leu Gly Ala Gly Ser Ser 390 395 Gln Asn Leu Ile Phe Tyr Asp Pro Ile Glu Val Ser Asn Ala Gly Val 410 Ser Val Ser Phe Asn Lys Glu Ala Asp Gln Thr Gly Ser Val Val Phe 420 425 Ser Gly Ala Thr Val Asn Ser Ala Asp Phe His Gln Arg Asn Leu Gln 440 Thr Lys Thr Pro Ala Pro Leu Thr Leu Ser Asn Gly Phe Leu Cys 1le 455 Glu Asp His Ala Gln Leu Thr Val Asn Arg Phe Thr Gln Thr Gly Gly 470 475 Vai Val Ser Leu Gly Asn Gly Ala Val Leu Ser Cys Tyr Lys Asn Gly 485 490 Thr Gly Asp Ser Ala Ser Asn Ala Ser Ile Thr Leu Lys His Ile Gly 505 Leu Asn Leu Ser Ser Ile Leu Lys Ser Gly Ala Glu Ile Pro Leu Leu 520 Trp Val Glu Pro Thr Asn Asn Ser Asn Asn Tyr Thr Ala Asp Thr Ala 535 Ala Thr Phe Ser Leu Ser Asp Val Lys Leu Ser Leu Ile Asp Asp Tyr 550 555 Gly Asn Ser Pro Tyr Glu Ser Thr Asp Leu Thr His Ala Leu Ser Ser 565 570 Gln Pro Met Leu Ser Ile Ser Glu Ala Ser Asp Asn Gln Leu Gln Ser 585 Glu Asn Ile Asp Phe Ser Gly Leu Asn Val Pro His Tyr Gly Trp Gln 600 605 Gly Leu Trp Thr Trp Gly Trp Ala Lys Thr Gln Asp Pro Glu Pro Ala 615 Ser Ser Ala Thr Ile Thr Asp Pro Gln Lys Ala Asn Arg Phe His Arg 630 Thr Leu Leu Leu Thr Trp Leu Pro Ala Gly Tyr Val Pro Ser Pro Lys 645 650 His Arg Ser Pro Leu Ile Ala Asn Thr Leu Trp Gly Asn Met Leu Leu 665 Ala Thr Glu Ser Leu Lys Asn Ser Ala Glu Leu Thr Pro Ser Gly His 680 Pro Phe Trp Gly Ile Thr Gly Gly Gly Leu Gly Met Met Val Tyr Gln 695 700 Asp Pro Arg Glu Asn His Pro Gly Phe His Met Arg Ser Ser Gly Tyr 710 715 Ser Ala Gly Met Ile Ala Gly Gln Thr His Thr Phe Ser Leu Lys Phe 725 730 Ser Gln Thr Tyr Thr Lys Leu Asn Glu Arg Tyr Ala Lys Asn Asn Val 740 745 Ser Ser Lys Asn Tyr Ser Cys Gln Gly Glu Met Leu Phe Ser Leu Gln

760 765 755 Glu Gly Phe Leu Leu Thr Lys Leu Val Gly Leu Tyr Ser Tyr Gly Asp His Asn Cys His His Phe Tyr Thr Gln Gly Glu Asn Leu Thr Ser Gln 785 790 ~ 795 800 Gly Thr Phe Arg Ser Gln Thr Met Gly Gly Ala Val Phe Phe Asp Leu 805 810 Pro Met Lys Pro Phe Gly Ser Thr His Ile Leu Thr Ala Pro Phe Leu 820 825 Gly Ala Leu Gly Ile Tyr Ser Ser Leu Ser His Phe Thr Glu Val Gly 840 Ala Tyr Pro Arg Ser Phe Ser Thr Lys Thr Pro Leu Ile Asn Val Leu 855 860 Val Pro Ile Gly Val Lys Gly Ser Phe Met Asn Ala Thr His Arg Pro 875 870 Gln Ala Trp Thr Val Glu Leu Ala Tyr Gln Pro Val Leu Tyr Arg Gln 885 8.90 Glu Pro Gly Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe 905 900 Gly Ser Gly Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser 920 Gln Gln Thr Gln Pro Leu Ser Trp Leu Thr Leu His Phe Gln Tyr His 930 935 940 Gly Phe Tyr Ser Ser Ser Thr Phe Cys Asn Tyr Leu Asn Gly Glu Ile 945 950 955 Ala Leu Arg Phe

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<212> PRT

<213> Chlamydia

<400> 178

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				169	5				17	^				1.7	-
Leu	ı Ile	e Phe	e Glu 180	Lys		E Lys	s Gly	/ Gly	y Le	u Gli	u Pho	e Al			s Sei
Ser	Le	1 Glu 199	ı Glı		/ Gly	/ Ala	a Cys	s Ala		a Gli	n Se	r Il		u Il	e His
Asp	Cys 210	s Glr		Leu	ı Glr	val 215	l Lys		в Суя	s Thi	r Thi	r Ala		l Ası	n Ala
Gl: 225	Gly	/ Ser	Sei	Ala	Asr 230	Asp		Leu	ı Gly	/ Phe	e Gly		y Gly	y Ala	a Phe 24(
Ph∈	· Val	Thr	Gly	/ Ser 245		Ser	Gly	/ Glu	ı Lys 250	s Sei		і Туі	r Met	259) Ala
			260)				265	5				270	e Gli	ı Gly
		275	5				280)				285	5		/ Lys
	290)				295	,				300)			Arg
305					310					315	,				Gln 320
				325					330)				335	Glu
			340					345					350)	Val
		355					360					365			Ala
	370					375					380			_	Asn
385					390					395					Gly 400
				405					410					415	Gly
			420					425					430		Cys
		435					440					445			Asp
	450					455					460			_	Thr
465					470					475					Phe 480
				485	Leu				490					495	_
			500		Thr			505					510	_	-
		515					520					525			Gly
	530				Gly	535					540				
545					Leu 550					555					560
				565	Gly				570					575	
			580		Val		•	585					590		
Glu	Glu	Glu 595	Ala	Thr	Leu	Leu	Gly 600	Cys	Cys	Gly	Gly	Gly 605	Ala	Val	His

Gly	Met 610	Asp	Ser	Thr	Ser	Ile 615	Val	Gly	Asn	Ser	Ser 620	Val	Arg	Phe	Gly
	Asn	Tyr	Ala	Meţ	Gly 630	Gln	Gly	Val	ser	Gly 635	Gly	Ala	Leu	Leu	Ser 640
625 Lys	Thr	Val	Gln		Ala	Gly	Asn	Gly	Ser 650		Asp	Phe	Ser	Arg 655	Asn
Ile	Ala	Ser		645 Gly	Gly	Gly	Ala			Ala	Ser	Glu	Gly 670		Cys
Glu	Leu	Val	660 Asp	Asn	Gly	Tyr	Val	665 Leu	Phe	Arg	Asp	Asn		Gly	Arg
	Tyr	675					680					685			,
	690					695					700				
705	Asn				710					715					720
_	Val			725					730					735	
Glu	Gln	Lys	Asp 740	Asn	Asn	Glu	Leu	Ser 745	Prie	Leu	Gly	Ser	Val 750	Glu	Gln
	Phe	755					760					765			
Asp	Leu 770	Ser	Pro	Glu	Ser	Ser 775	Ile	Ser	Ser	Glu	Glu 780	Leu	Ala	Lys	Arg
	Glu	Cys	Ala	Gly	Gly 790	Ala	Ile	Phe	Ala	Lys 795	Arg	Val	Arg	Ile	Val 800
785 Asp	Asn	Gln	Glu			Val	Phe	Ser	Asn 810		Phe	Ser	Asp	Ile 815	
Gly	Gly	Ala		805 Phe	Thr	G_y	Ser	Leu 825		Glu	Glu	Asp	Lys 830		Asp
Gly	Gln		820 Pro	Glu	Val	Leu	Ile 840		Gly	Asn	Ala	Gly 845		Val	Val
Phe	Ser	835 Gly	Asn	Ser	Ser	Lys 855		Asp	Glu	His	Leu 860		His	Thr	Gly
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865 Asn	Val	Leu	Phe	Tyr	Asn	Asn	Val	Ala	Cys	Ser	Gly	Gly	Ala	Val	
Ile	Glu	Asp	His	885 Gly	Asn	Val	Leu	Leu	890 Glu		Phe	Gly	Gly	895 Asp	Ile
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	· Phe	915					920					925			
	930					935					940				
945	His				950					955					960
Glu	Arg	Lys	Ser	Ala 965		Val	Leu	Leu	. Ile 970	Asn	Ser	Arg	Glu	Asn 975	Pro
Gly	y Tyr	Thr	Gly 980	Ser	Ile	Arg	Phe	Leu 985	Glu	Ala	Glu	Ser	Lys 990	Val	Pro
Glr	Cys	Ile 995	His	Val	Gln	Gln	Gly 100		Leu	Glu	Leu	Leu 100	Asn 5	Gly	Ala
Thr	Leu 101	Cys	Ser	Tyr	Gly	Phe	Lys	Gln	Asp	Ala	Gly 102	Ala 0	Lys	Leu	Val
	ı Ala	Ala	Gly	Ser		Leu	Lys	Ile	Leu	Asp	Ser 5	Gly	Thr	Pro	Val 1040
102 Glr	s 1 Gly	His	Ala	Ile	103 Ser	u Lys	Pro	Glu	Ala			Glu	Ser	Ser	Ser

		1045				105	0				105	55
Glu Pro		y Ala Hi 60	s Ser	Leu	Trp 106		Ala	Lys	Asn	Ala 107	Glr	
Thr Val	Pro Me 1075	t Val As	p Ile	His 1080		Ile	Ser	Val	Asp		Ala	Ser
Phe Ser 109		r Gln Gl	n Glu 1095		Thr	Val	Glu	Ala 110		Gln	Val	Ile
Val Pro 1105	Gly Gl	y Ser Ty 11		Arg	Ser	Gly	Glu 111		Asn	Leu	Glu	Leu 1120
Val Asn	Thr Th	r Gly Th 1125	r Gly	Tyr	Glu	Asn 113		Ala	Leu	Leu	Lys 113	Asn
	11	l Pro Le			1145	5				115	כ	
	1155	e Ser As:		1160)				116	5		
117	0	u Ile Gl	1175	5				118	0			=
1185		s Ile Gla	90				119	5				1200
		g Leu Ası 1205				1210)				121	5
	12:	_ •			1225	· •				1230)	_
	1235	n Leu Thi		1240)				1245	5		
125	0	y Phe Ala	1255	;				1260)			
1265		a Ile Ası 127	70				1275	5				1280
		p Ile Glr 1285				1290)				129	5
	130				1305					1310		
	1315	s Gly Val		1320					1325	;		
1330	כ	e Phe Lys	1335					1340	1			
1345		r Arg Tyr 135	0				1355	,				1360
		/ Val Leu 1365				1370				_	1375	5
	138				1385					1390		-
	1395	Tyr Ala	:	1400					1405			
Gly Arg 1410		a Arg Ser	Phe (Asp .	Ala		Leu 1420		Asn	Ile	Thr
Ile Pro 1425	Leu Gly	Met Lys 143		Glu 1	Leu .		Phe 1435		Lys	Gly	Gln	Phe 1440
Ser Glu	Val Asr	Ser Leu 1445	Gly :	Ile S		Tyr 1450		Trp	Glu		Tyr 1455	_
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Glu Gly	Ala Pro 1475	Met Asp		Pro <i>1</i> 1480	Arg (Gln (Glu		Arg 1485	Val .	Ala	Leu

Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe Ser Thr Val Leu Gly Leu 1500 1495 Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr Asp Ser Lys Leu Gly Tyr 1505 1510 1515 1520 1510 Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe 1525 <210> 179

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325

MadMadida ettaMishkisteisteinkalasea ihkistii tae vara vaan ka ejen ehe ett joodija asija asija ja ja ja ja ethiseilistiit

Ile Thr Gly Ile Thr Gly Thr Ile Asp Phe Val Ser Asn Ile Ala Thr 345 Asp Ser Gly Ala Gly Val Phe Thr Lys Glu Asn Leu Ser Cys Thr Asn 360 Thr Asn Ser Leu Gln Phe Leu Lys Asn Ser Ala Gly Gln His Gly Gly 375 Gly Ala Tyr Val Thr Gln Thr Met Ser Val Thr Asn Thr Thr Ser Glu 390 395 Ser Ile Thr Thr Pro Pro Leu Val Gly Glu Val Ile Phe Ser Glu Asn 405 410 Thr Ala Lys Gly His Gly Gly Gly Ile Cys Thr Asn Lys Leu Ser Leu 420 425 Ser Asn Leu Lys Thr Val Thr Leu Thr Lys Asn Ser Ala Lys Glu Ser 440 445 Gly Gly Ala Ile Phe Thr Asp Leu Ala Ser Ile Pro Thr Thr Asp Thr 455 460 Pro Glu Ser Ser Thr Pro Ser Ser Ser Pro Ala Ser Thr Pro Glu 470 475 Val Val Ala Ser Ala Lys Ile Asn Arg Phe Phe Ala Ser Thr Ala Glu 485 490 Pro Ala Ala Fro Ser Leu Thr Glu Ala Glu Ser Asp Gln Thr Asp Gln 505 Thr Glu Thr Ser Asp Thr Asn Ser Asp Ile Asp Val Ser Ile Glu Asn 520 525 Ile Leu Asn Val Ala Ile Asn Gln Asn Thr Ser Ala Lys Lys Gly Gly 535 540 Ala Ile Tyr Gly Lys Lys Ala Lys Leu Ser Arg Ile Asn Asn Leu Glu 550 555 Leu Ser Gly Asn Ser Ser Gln Asp Val Gly Gly Leu Cys Leu Thr 565 570 Glu Ser Val Glu Phe Asp Ala Ile Gly Ser Leu Leu Ser His Tyr Asn 585 Ser Ala Ala Lys Glu Gly Gly Val Ile His Ser Lys Thr Val Thr Leu 600 605 Ser Asn Leu Lys Ser Thr Phe Thr Phe Ala Asp Asn Thr Val Lys Ala 615 620 Ile Val Glu Ser Thr Pro Glu Ala Pro Glu Glu Ile Pro Pro Val Glu 635 Gly Glu Glu Ser Thr Ala Thr Glu Asn Pro Asn Ser Asn Thr Glu Gly 645 650 Ser Ser Ala Asn Thr Asn Leu Glu Gly Ser Gln Gly Asp Thr Ala Asp 660 665 Thr Gly Thr Gly Val Val Asn Asn Glu Ser Gln Asp Thr Ser Asp Thr 680 Gly Asn Ala Glu Ser Gly Glu Gln Leu Gln Asp Ser Thr Gln Ser Asn 695 Glu Glu Asn Thr Leu Pro Asn Ser Ser Ile Asp Gln Ser Asn Glu Asn 710 715 Thr Asp Glu Ser Ser Asp Ser His Thr Glu Glu Ile Thr Asp Glu Ser 725 730 Val Ser Ser Ser Ser Lys Ser Gly Ser Ser Thr Pro Gln Asp Gly Gly 745 Ala Ala Ser Ser Gly Ala Pro Ser Gly Asp Gln Ser Ile Ser Ala Asn 760 Ala Cys Leu Ala Lys Ser Tyr Ala Ala Ser Thr Asp Ser Ser Pro Val

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		Ser	Ser	GIA	790	ASP	VAI	1111	1114	795					800
785	 C 0 20	-		Clv	Agn	Ser	Δla	giv"	Asp	Ser	Glū	Gly	Pro	Thr	Glu
Ser	Ser	261	SEL	805	дал	501		017	810			-		815	
Dro	Glu	Δla	Glv	Ser	Thr	Thr	Glu	Thr		Thr	Leu	Ile	Gly	Gly	${ t Gly}$
			820					825					830		
Δla	Tle	Tvr	Glv	Glu	Thr	Val	Lys	Ile	Glu	Asn	Phe	Ser	Gly	Gln	Gly
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Ile	Phe	Ser	Gly	Asn	Lys	Ala	Ile	Asp	Asn	Thr	Thr	Glu	${ t Gly}$	Ser	Ser
	950					855					860				
Ser	Lvs	Ser	Asn	Val	Leu	Gly	Gly	Ala	Val	Tyr	Ala	Lys	Thr	Leu	Phe
865					870					875					000
Asn	Leu	Asp	Ser	${\tt Gly}$	Ser	Ser	Arg	Arg	Thr	Val	Thr	Phe	Ser	Gly	Asn
				885					890					895	
Thr	Val	Ser	Ser	Gln	Ser	Thr	Thr	Gly	Gln	Val	Ala	GIY	Gly	Ala	шe
			900					905			** - 1	Dha	910	Tura	Acn
Tyr	Ser	Pro	Thr	Val	Thr	Ile		Thr	Pro	Val	Val	Pne	Ser	гуѕ	ASII
		915			- -	_	920		rm la sa	200	The	925	λrα	LVC	Asn
Ser			Asn	Asn	Ala		Asn	Ala	LIII	Asp	940	GIII	Arg	цуз	, and
	930		~3	n 1 -	T] a	935	הוג	T'h~	Sar	Ala		Ser	Leu	Ser	Glv
	Phe	GIY	GIY	Ala	950	GIY	Ala	1111	361	955	V-3.1	00-			960
945	21.	III a	Dho	T 011	950 Glu	Δen	Val	Ala	Asp	Leu	Glv	Ser	Ala	Ile	Gly
GIY	Ald	nıs	PHE	965	Olu	71.511	• • • •	****	970		- 1			975	
Leu	Val	Pro	Asp	Thr	Gln	Asn	Thr	Glu	Thr	Val	Lys	Leu	Glu	Ser	Gly
			980					985					990		
Ser	Tvr	īvr	Phe	Glu	Lys	Asn	Lys	Ala	Leu	Lys	Arg	Ala	Thr	Ile	Tyr
		995					100	0				100	5		
Ala	Pro	Val	Val	Ser	Ile	Lys	Ala	Tyr	Thr	Ala	Thr	Phe	Asn	Gln	Asn
	101	Λ				101	5				102	0			
Arg	Ser	Leu	Glu	Glu	Gly	Ser	Ala	Ile	Tyr	Phe	Thr	Lys	Glu	Ala	ser
102	5				103	0				103			**- 7	mb so	1040
Ile	Glu	Ser	Leu			Val	Leu	Phe	Thr	Gly	Asn	ьeu	vaı	105	E PIO
				104	5		~ 7	m1	105		The	mb x	cor		
Thr	Leu	Ser			Thr	GIu	Gly	inr	Pro	Ala	TILL	1111	107	U U	rap
_		_	106	0 ~7		21-	т1 ^	106		. Cln	Tle	Δla			Asn
Val	Thr			. GIA	Ala	Ата	108	n n	Gry	GIII	. 110	108	5	002	Asn
~3		107	/ 5 	. 7.00	, han	Lou			Lvs	Len	Tle			Glv	Gly
GIY			i Thi	ASL	ASII	109	, F10	шси	шy	, 100	110	0			-
200	109	, C.r.	Dhe) Arc	λαη			Tvr	Arc	r Pro			Ser	Asp	Thr
		: Cya) File	, Arg	111		. 010			111	.5			_	1120
110	/O / Thi	· Car	- Thr	· Phe	Cvs	Ser	· Ile	Ala	Glv	Asp	Val	Lys	Leu	Thr	Met
GLY	1111	. 501	. 1111	112	. 0,10 !5				113	30		-		113	5
Glr	. Ala	a Ala	a Tivs	Glv	Lvs	Thr	Ile	Ser	Phe	e Phe	Asp	Ala	Ile	Arg	Thr
			114	10				114	5				115	0	
Ser	Th	r Lvs	s Lvs	Thr	Gly	Thr	Glr	a Ala	Thi	. Ala	Туг	Asp	Thr	Leu	Asp
		119	55				116	50				116	5		
Ile	Ası	ı Lys	s Sei	Glu	ı Asp	Ser	Glu	ı Thr	Va.	l Asr	ı Ser	Ala	Phe	Thr	Gly
	111	70				117	75				118	30			
Thi	c Ile	e Lei	ı Phe	e Sei	Ser	Glu	ı Let	ı His	Glı	ı Asr	Lys	Ser	Туг	Ile	Pro
118	35				119	90				119	95				1200
Gl	n Ası	n Va	l Va	l Lei	ı His	s Ser	Gly	/ Ser	Le	ı Val	Leu	ı Lys	Pro	ASE	Thr
				120)5				12:	τO				121	

kalan kurna mangita penggapanga kang mga taga haling kang kang kang kang mga mga mga mga mga mga mga mga mga m

			122	0				122	5				123	0	val
Met -	Thr	Pro 123	Gly 5	Ser	Val	Leu	Ser 124		Gln	Thr	Val	Ala 124	_	Gly	Ala
Leu	Val 125		Asn	Asn	Met	Thr 125		Asp	Leu	Ser	Ser 126	Val		Lys	Asn
		Ala	Glu	Gly	Asn			Thr	Pro	Pro			Arg	Ile	Ile
126	_			_	127					127					1280
Asp	Thr	Thr	Thr	Ser 128		Ser	Gly	Gly			Ser	Thr	Asp		
Ser	Asn	Gln	Asn		_	Asp	Thr	Lvs	129		λen	λαπ	Asn	129	5 315
			130					130		0111	ASII	ASII	131		MIG
Ser	Asn	Gln	Gly	Glu	Ser	Ala	Asn	Gly	Ser	Ser	Ser	Pro	Ala	Val	Ala
		131	5				1320	2				132	5		
Ala	133		Thr	Ser	Arg	Thṛ 133!		Asn	Phe	Ala			Ala	Thr	Ala
Thr			Thr	Thr	Pro			Thr	Thr	Thr	134	U Sar	Asn	Gla	1751
134	5				135	0				135	5				1360
Ile	Leu	Gly	Gly	Glu	Ile	Lys	Leu	Ile	Asp			Gly	Thr	Phe	Phe
				136	5				137	0				137	5
Gin	Asn	Pro			Arg	Ser	Asp			Ile	Ser	Leu	Leu		Leu
Pro	Thr	Asn	1380 Ser		Laze	Mot	Gln	1385		Tura	T10	17-1	139 Leu		03
		1395		301	цуз	MEC	1400		GIII	ьуѕ	1.te	140		inr	GIY
Asp	Ile	Ala	Pro	Gln	Lys	Gly			Gly	Thr	Leu		Leu	Asp	Pro
	141	0				1415	5				142	J		_	
		Leu	Gln	Asn			Ile	Ser	Ala			Lys	Phe	Asp	Ser
1429		<i>C</i> 15	TT	71.	1430		D	•	_	1435					1440
тут	Arg	GIH	пр	1445		vai	Pro	Arg	1450		Hıs	Phe	Tyr		
Ser	Tle	Leu	Glv			Mot	Ser	Mer			Val	Lvs	Gln	145!	T.e.i
				\sim \sim \sim		Met.						-1-			DC G
			1460)				1465	;				1470)	
		Asp	1460 Lys)			Ala	1465 Arg	;			Val)	Asn
Leu	Asn	Asp 1475	1460 Lys) Met	Asn	Leu	Ala 1480	1465 Arg	Phe	Asp	Glu	1489	1470 Ser) Tyr	
Leu	Asn Leu	Asp 1475 Trp	1460 Lys) Met	Asn	Leu Leu	Ala 1480 Gly	1465 Arg	Phe	Asp	Glu Ser	1485 Gln	1470 Ser) Tyr	
Leu Asn	Asn Leu 1490	Asp 1475 Trp)	1460 Lys Ile	Met Ser	Asn Gly	Leu Leu 1495	Ala 1480 Gly	1465 Arg Thr	Phe Met	Asp Leu	Glu Ser 1500	1485 Gln)	1470 Ser Val	Tyr Gly	Thr
Leu Asn	Asn Leu 1490 Thr	Asp 1475 Trp)	1460 Lys Ile	Met Ser	Asn Gly	Leu Leu 1495 Thr	Ala 1480 Gly	1465 Arg Thr	Phe Met	Asp Leu	Glu Ser 1500 Gly	1485 Gln)	1470 Ser	Tyr Gly	Thr Ala
Leu Asn Pro	Asn Leu 1490 Thr	Asp 1475 Trp) Ser	1460 Lys Ile Glu	Met Ser Glu	Asn Gly Phe 1510	Leu Leu 1495 Thr	Ala 1480 Gly Tyr	1465 Arg Thr	Phe Met Ser	Asp Leu Arg 1515	Glu Ser 1500 Gly	1489 Gln) Ala	1470 Ser Val	Tyr Gly Val	Thr Ala 1520
Leu Asn Pro 1505 Leu	Asn Leu 1490 Thr Asp	Asp 1475 Trp) Ser Ala	1460 Lys Ile Glu Lys	Met Ser Glu Pro 1525	Asn Gly Phe 1510 Ala	Leu Leu 1495 Thr His	Ala 1480 Gly Tyr Asp	1465 Arg Thr Tyr Val	Phe Met Ser Ile 1530	Asp Leu Arg 1515 Val	Glu Ser 1500 Gly Gly	1489 Gln) Ala Ala	1470 Ser Val Ser Ala	Tyr Gly Val Phe	Thr Ala 1520 Ser
Leu Asn Pro 1505 Leu	Asn Leu 1490 Thr Asp	Asp 1475 Trp Ser Ala	1460 Lys Ile Glu Lys Gly	Met Ser Glu Pro 1525 Lys	Asn Gly Phe 1510 Ala	Leu Leu 1495 Thr His	Ala 1480 Gly Tyr Asp	1465 Arg Thr Tyr Val Leu	Phe Met Ser Ile 1530 Lys	Asp Leu Arg 1515 Val	Glu Ser 1500 Gly Gly	1489 Gln) Ala Ala	1470 Ser Val Ser Ala	Tyr Gly Val Phe 1535	Thr Ala 1520 Ser
Leu Asn Pro 1505 Leu Lys	Asn Leu 1490 Thr Asp Met	Asp 1475 Trp) Ser Ala	1460 Lys Ile Glu Lys Gly 1540	Met Ser Glu Pro 1525 Lys	Asn Gly Phe 1510 Ala	Leu Leu 1495 Thr His	Ala 1480 Gly Tyr Asp	1465 Arg Thr Tyr Val Leu 1545	Phe Met Ser Ile 1530 Lys	Asp Leu Arg 1515 Val Arg	Glu Ser 1500 Gly Gly Gly	1485 Gln Ala Ala Asn	1470 Ser Val Ser Ala Asn	Tyr Gly Val Phe 1535	Thr Ala 1520 Ser Thr
Leu Asn Pro 1505 Leu Lys	Asn Leu 1490 Thr Asp Met	Asp 1475 Trp) Ser Ala Ile	1460 Lys Ile Glu Lys Gly 1540 Ser	Met Ser Glu Pro 1525 Lys	Asn Gly Phe 1510 Ala	Leu Leu 1495 Thr His Lys	Ala 1480 Gly Tyr Asp Ser	1465 Arg Thr Tyr Val Leu 1545 Gln	Phe Met Ser Ile 1530 Lys	Asp Leu Arg 1515 Val Arg	Glu Ser 1500 Gly Gly Gly	1489 Gln Ala Ala Asn Tyr	1470 Ser Val Ser Ala Asn 1550 Gly	Tyr Gly Val Phe 1535	Thr Ala 1520 Ser Thr
Leu Asn Pro 1505 Leu Lys His	Leu 1490 Thr Asp Met	Asp 1475 Trp) Ser Ala Ile Gly 1555	1460 Lys Ile Glu Lys Gly 1540 Ser	Met Ser Glu Pro 1525 Lys	Asn Gly Phe 1510 Ala Thr	Leu Leu 1495 Thr His Lys	Ala 1480 Gly Tyr Asp Ser Tyr 1560	1465 Arg Thr Tyr Val Leu 1545 Gln	Phe Met Ser Ile 1530 Lys Ala	Asp Ieu Arg 1515 Val Arg	Glu Ser 1500 Gly Gly Glu Val	Gln Ala Ala Asn Tyr 1565	Ser Val Ser Ala Asn 1550 Gly	Tyr Gly Val Phe 1535 Tyr Gly	Thr Ala 1520 Ser Thr
Leu Asn Pro 1505 Leu Lys His	Leu 1490 Thr Asp Met Lys Phe 1570	Asp 1475 Trp Ser Ala Ile Gly 1555 His	1460 Lys Ile Glu Lys Gly 1540 Ser	Met Ser Glu Pro 1525 Lys	Asn Gly Phe 1510 Ala Thr Tyr	Leu Leu 1495 Thr His Lys Ser Asn	Ala 1480 Gly Tyr Asp Ser Tyr 1560 Lys	1465 Arg Thr Tyr Val Leu 1545 Gln	Phe Met Ser Ile 1530 Lys Ala	Asp Leu Arg 1515 Val Arg Ser	Glu Ser 1500 Gly Gly Glu Val Lys 1580	Ala Ala Asn Tyr 1565 Ser	Ser Val Ser Ala Asn 1550 Gly Leu	Tyr Gly Val Phe 1535 Tyr Gly Pro	Thr Ala 1520 Ser Thr Lys
Leu Asn Pro 1505 Leu Lys His Pro	Leu 1490 Thr Asp Met Lys Phe 1570 Leu	Asp 1475 Trp Ser Ala Ile Gly 1555 His	1460 Lys Ile Glu Lys Gly 1540 Ser	Met Ser Glu Pro 1525 Lys Glu Val	Asn Gly Phe 1510 Ala Thr Tyr Ile	Leu 1495 Thr His Lys Ser Asn 1575 Ser	Ala 1480 Gly Tyr Asp Ser Tyr 1560 Lys	1465 Arg Thr Tyr Val Leu 1545 Gln	Phe Met Ser Ile 1530 Lys Ala	Asp Leu Arg 1515 Val Arg Ser	Glu Ser 1500 Gly Gly Glu Val Lys 1580	Ala Ala Asn Tyr 1565 Ser	Ser Val Ser Ala Asn 1550 Gly	Tyr Gly Val Phe 1535 Tyr Gly Pro	Thr Ala 1520 Ser Thr Lys
Leu Asn Pro 1505 Leu Lys His Pro Leu 1585	Leu 1490 Thr Asp Met Lys Phe 1570 Leu	Asp 1475 Trp Ser Ala Ile Gly 1555 His	1460 Lys Ile Glu Lys Gly 1540 Ser Phe	Met Ser Glu Pro 1525 Lys Glu Val	Asn Gly Phe 1510 Ala Thr Tyr Ile Ile 1590	Leu 1495 Thr His Lys Ser Asn 1575 Ser	Ala 1480 Gly Tyr Asp Ser Tyr 1560 Lys	1465 Arg Thr Tyr Val Leu 1545 Gln Lys	Phe Met Ser Ile 1530 Lys Ala Thr	Asp Ieu Arg 1515 Val Arg Ser Glu Ile 1595	Glu Ser 1500 Gly Gly Glu Val Lys 1580 Lys	1485 Gln Ala Ala Asn Tyr 1565 Ser	Ser Val Ser Ala Asn 1550 Gly Leu Asp	Tyr Gly Val Phe 1535 Tyr Gly Pro	Thr Ala 1520 Ser Thr Lys Leu Val
Leu Asn Pro 1505 Leu Lys His Pro Leu 1585	Leu 1490 Thr Asp Met Lys Phe 1570 Leu	Asp 1475 Trp Ser Ala Ile Gly 1555 His	1460 Lys Ile Glu Lys Gly 1540 Ser Phe Gly	Met Ser Glu Pro 1525 Lys Glu Val Val	Asn Gly Phe 1510 Ala Thr Tyr Ile Ile 1590 Ile	Leu 1495 Thr His Lys Ser Asn 1575 Ser	Ala 1480 Gly Tyr Asp Ser Tyr 1560 Lys	1465 Arg Thr Tyr Val Leu 1545 Gln Lys Gly	Phe Met Ser Ile 1530 Lys Ala Thr Tyr	Asp Leu Arg 1515 Val Arg Ser Glu Ile 1595 Gln	Glu Ser 1500 Gly Gly Glu Val Lys 1580 Lys	1485 Gln Ala Ala Asn Tyr 1565 Ser	1470 Ser Val Ser Ala Asn 1550 Gly Leu Asp	Tyr Gly Val Phe 1535 Tyr Gly Pro Thr	Thr Ala 1520 Ser Thr Lys Leu Val 1600 Asp
Leu Asn Pro 1505 Leu Lys His Pro Leu 1585 Thr	Leu 1490 Thr Asp Met Lys Phe 1570 Leu	Asp 1475 Trp Ser Ala Ile Gly 1555 His Gln	1460 Lys Ile Glu Lys Gly 1540 Ser Phe Gly	Met Ser Glu Pro 1525 Lys Glu Val Val Thr 1605	Asn Gly Phe 1510 Ala Thr Tyr Ile 1590 Ile	Leu 1495 Thr His Lys Ser Asn 1575 Ser	Ala 1480 Gly Tyr Asp Ser Tyr 1560 Lys	1465 Arg Thr Tyr Val Leu 1545 Gln Lys Gly Arg	Phe Met Ser Ile 1530 Lys Ala Thr Tyr Asn 1610	Asp Ieu Arg 1515 Val Arg Ser Glu Ile 1595 Gln	Ser 1500 Gly Gly Glu Val Lys 1580 Lys	1485 Gln Ala Ala Asn Tyr 1565 Ser His	1470 Ser Val Ser Ala Asn 1550 Gly Leu Asp	Tyr Gly Val Phe 1535 Tyr Gly Pro Thr Glu 1615	Thr Ala 1520 Ser Thr Lys Leu Val 1600 Asp
Leu Asn Pro 1505 Leu Lys His Pro Leu 1585 Thr	Leu 1490 Thr Asp Met Lys Phe 1570 Leu	Asp 1475 Trp Ser Ala Ile Gly 1555 His Gln Tyr	1460 Lys Ile Glu Lys Gly 1540 Ser Phe Gly	Met Ser Glu Pro 1525 Lys Glu Val Val Thr 1605	Asn Gly Phe 1510 Ala Thr Tyr Ile 1590 Ile	Leu 1495 Thr His Lys Ser Asn 1575 Ser	Ala 1480 Gly Tyr Asp Ser Tyr 1560 Lys Tyr	1465 Arg Thr Tyr Val Leu 1545 Gln Lys Gly Arg	Phe Met Ser Ile 1530 Lys Ala Thr Tyr Asn 1610	Asp Ieu Arg 1515 Val Arg Ser Glu Ile 1595 Gln	Ser 1500 Gly Gly Glu Val Lys 1580 Lys	1485 Gln Ala Ala Asn Tyr 1565 Ser His	1470 Ser Val Ser Ala Asn 1550 Gly Leu Asp Trp	Tyr Gly Val Phe 1535 Tyr Gly Pro Thr Glu 1615	Thr Ala 1520 Ser Thr Lys Leu Val 1600 Asp
Leu Asn Pro 1505 Leu Lys His Pro Leu 1585 Thr	Leu 1490 Thr Asp Met Lys Phe 1570 Leu His	Asp 1475 Trp Ser Ala Ile Gly 1555 His Gln Tyr	1460 Lys Ile Glu Lys Gly 1540 Ser Phe Gly Pro Leu 1620	Met Ser Glu Pro 1525 Lys Glu Val Thr 1605	Asn Gly Phe 1510 Ala Thr Tyr Ile 1590 Ile Ala	Leu 1495 Thr His Lys Ser Asn 1575 Ser Arg	Ala 1480 Gly Tyr Asp Ser Tyr 1560 Lys Tyr Glu	1465 Arg Thr Tyr Val Leu 1545 Gln Lys Gly Arg	Phe Met Ser Ile 1530 Lys Ala Thr Tyr Asn 1610 Ser	Asp Ieu Arg 1515 Val Arg Ser Glu Ile 1595 Gln	Ser 1500 Gly Gly Glu Val Lys 1580 Lys Gly	1485 Gln Ala Ala Asn Tyr 1565 Ser His Glu Leu	1470 Ser Val Ser Ala Asn 1550 Gly Leu Asp Trp Arg 1630	Tyr Gly Val Phe 1535 Tyr Gly Pro Thr Glu 1615	Thr Ala 1520 Ser Thr Lys Leu Val 1600 Asp
Leu Asn Pro 1505 Leu Lys His Pro Leu 1585 Thr Leu Ala	Leu 1490 Thr Asp Met Lys Phe 1570 Leu His Gly	Asp 1475 Trp Ser Ala Ile Gly 1555 His Gln Tyr Trp	1460 Lys Ile Glu Lys Ser Phe Gly Pro Leu 1620 Asp	Met Ser Glu Pro 1525 Lys Glu Val Thr 1605 Thr	Asn Gly Phe 1510 Ala Thr Tyr Ile 1590 Ile Ala Lys	Leu 1495 Thr His Lys Ser Asn 1575 Ser Arg Leu Arg	Ala 1480 Gly Tyr Asp Ser Tyr 1560 Lys Tyr Glu Arg	Thr Tyr Val Leu 1545 Gln Lys Gly Arg Val 1625 Thr	Phe Met Ser Ile 1530 Lys Ala Thr Tyr Asn 1610 Ser Val	Asp Leu Arg 1515 Val Arg Ser Glu Ile 1595 Gln Ser	Ser 1500 Gly Gly Glu Val Lys 1580 Lys Gly Val	1485 Gln Ala Ala Asn Tyr 1565 Ser His Glu Leu Glu 1645	Ser Val Ser Ala Asn 1550 Gly Leu Asp Trp Arg 1630 Leu	Tyr Gly Val Phe 1535 Tyr Gly Pro Thr Glu 1615 Thr	Thr Ala 1520 Ser Thr Lys Leu Val 1600 Asp Pro

1660 1655 Tyr Phe Asp Asn Cys Thr Tyr Arg Asn Leu Ala Ile Pro Met Gly Leu 1665 _ 1670 _ 1675 Ala Phe Glu Gly Glu Leu Ser Gly Asn Asp Ile Leu Met Tyr Asn Arg 1685 1690 1695 Phe Ser Val Ala Tyr Met Pro Ser Ile Tyr Arg Asn Ser Pro Thr Cys 1700 1705 1710 Lys Tyr Gln Val Leu Ser Ser Gly Glu Gly Gly Glu Ile Ile Cys Gly 1715 1720 1725 Val Fro Thr Arg Asn Ser Ala Arg Gly Glu Tyr Ser Thr Gln Leu Tyr 1730 1735 1740 Pro Gly Pro Leu Trp Thr Leu Tyr Gly Ser Tyr Thr Ile Glu Ala Asp 1745 1750 1755 Ala His Thr Leu Ala His Met Met Asn Cys Gly Ala Arg Met Thr Phe 1**7**70 1765 <210> 180 <211> 1752 <212> PRT <213> Chlamydia <400> 180 Met Lys Trp Leu Ser Ala Thr Ala Val Phe Ala Ala Val Leu Pro Ser 10 Val Ser Gly Phe Cys Phe Pro Glu Pro Lys Glu Leu Asn Phe Ser Arg 25 Val Glu Thr Ser Ser Ser Thr Thr Phe Thr Glu Thr Ile Gly Glu Ala 40 Gly Ala Glu Tyr Ile Val Ser Gly Asn Ala Ser Phe Thr Lys Phe Thr 55 60 Asn Ile Pro Thr Thr Asp Thr Thr Thr Pro Thr Asn Ser Asn Ser Ser 75 70 Ser Ser Ser Gly Glu Thr Ala Ser Val Ser Glu Asp Ser Asp Ser Thr 90 Thr Thr Thr Pro Asp Pro Lys Gly Gly Gly Ala Phe Tyr Asn Ala His 105 100 Ser Gly Val Leu Ser Phe Met Thr Arg Ser Gly Thr Glu Gly Ser Leu 120 115 Thr Leu Ser Glu Ile Lys Met Thr Gly Glu Gly Gly Ala Ile Phe Ser 130 135 140 Gln Gly Glu Leu Leu Phe Thr Asp Leu Thr Ser Leu Thr Ile Gln Asn 155 150 Asn Leu Ser Gln Leu Ser Gly Gly Ala Ile Phe Gly Gly Ser Thr Ile 170 Ser Leu Ser Gly Ile Thr Lys Ala Thr Phe Ser Cys Asn Ser Ala Glu 180 185 Val Pro Ala Pro Val Lys Lys Pro Thr Glu Pro Lys Ala Gln Thr Ala 195 200 205 Ser Glu Thr Ser Gly Ser Ser Ser Ser Gly Asn Asp Ser Val Ser 220 210 215 Ser Pro Ser Ser Ser Arg Ala Glu Pro Ala Ala Ala Asn Leu Gln Ser 235 230 His Phe Ile Cys Ala Thr Ala Thr Pro Ala Ala Gln Thr Asp Thr Glu 250

Thr Ser Thr Pro Ser His Lys Pro Gly Ser Gly Gly Ala Ile Tyr Ala

nadalih kadalan katalan kan diftan diftan dipangan kan pagaj ga mengaj amanan kejan pada kan baha kan kali na h Kan

			260)				265	5				270)	
		275	•				280	Ser	Glr			285	Phe	e Sei	: Ile
	290	1				295					300)			Val
305					310					315	,				320
				325					330)				335	
			340				•	345					350)	Gly
		355					360					365			Ile
	370					375					380				Thr
385					390					395					Trp 400
				405					410					415	
•			420					425					430		Val.
		435			Gly		440			: :		445			
	450				Gly	455					460				
465					Ala 470				-	475					480
				485	Phe				490					495	_
			500		Asp			505					510		=
		515			Asn		520	• •				525			
	530				Lys	535					540				
545					Thr 550					555					560
				565	Gln				570					575	
			580		His			585					590		
		595			Gly		600					605			
	610					615					620				
625					Cys 630					635					640
				645	Ala				650					655	
			660		Leu			665					670		
		675			Ala		680					685			
Lys	Glu 690	Thr	Gln	Asp	Pro .	Asn 695	Ala	Asp	Thr	Asp	Leu 700	Leu	Ile	Asp	Tyr

Val 705	Val	Asp	Thr	Thr	Ile 710	Ser	Lys	Asn	Thr	Ala 715	Lys	Lys	Gly	Gly	Gly 720
	Tyr	Ala	Lys	Lys 725	Ala	Lys	Met	Ser	Arg.		Asp	Gln	Leu	.Asn 735	
Ser	Glu	Asn	Ser 740		Thr	Glu	Ile	Gly 745		Gly	Ile	Cys	Cys 750		Glu
Ser	Leu	Glu 755		Asp	Ala	Leu	Val 760		Leu	Ser	Val	Thr 765	_	Asn	Leu
Val	Gly 770		Glu	Gly	Gly	Gly 775		His	Ala	Lys	Thr 780		Asn	Ile	Ser
Asn 785		Lys	Ser	Gly	Phe 790		Phe	Ser	Asn	Asr. 795			Asn	Ser	Ser 800
	Thr	Gly	Val	Ala 805	Thr	Thr	Ala	Ser	Ala 810	Pro	Ala	Ala	Ala	Ala 815	Ala
Ser	Leu	Gln	Ala 820	Ala	Ala	Ala	Ala	Ala 825	Pro	Ser	Ser	Pro	Ala 830	Thr	Pro
Thr	Tyr	Ser 835	Gly	Val	Val	Gly	Gly 840	Ala	Iìe	Tyr	Gly	Glu 845	Lys	Val	Thr
Phe	Ser 850	Gln	Cys	Ser	Gly	Thr 855	Cys	Gln	Phe	·Ser	Gly 860	Asn	Gln	Ala	Ile
Asp 865	Asn	Asn	Pro	Ser	Gln 870	Ser	Ser	Leu	Asn	Val 875	Gln	Gly	Gly	Ala	Ile 880
Tyr	Ala	Lys	Thr	Ser 885	Leu	Ser	Ile	Gly	Ser 890	Ser	Asp	Ala	Gly	Thr 895	Ser
			900	-	Asn			905		_	_		910		
		915			Gly		92C					925			
-	930				Ser	935					940				_
945				_	Gly 950					955					960
	_			965	Gly				970					975	
		_	980		Ala			985					990		
		995			Ser		1000)				1005	5		
	1010)			Glu	1015	5				1020)			
1025		ьуѕ	Arg	GIY	Ala 1030		Tyr	ser	Pro	1035		Ser	iie	гуѕ	1040
		Ile	Thr	Phe	Asn		Asn	Thr	Ser			Asp	Glv	Ser	
				1045					1050)				1055	5
	_		1060) -	_			1065	5			_	1070)	
		1075	5		Val		1080)				1085	5		
	1090)			Thr	1095	5				1100)			
		inr	inr	GIN	Ser		GIN	ınr	Asp			டeu	ınr	ьeu	
1105 Ala		Ser	Gly		1110 Ile		Phe	Ser				Leu	Gln		
Gln	Gly	Asp	Thr	1125 Pro	Ala	Ser	Lys	Phe	1130 Cys		Ile	Ala	Gly	1135 Tyr	

			114					114							
Lys	Leu	Ser 115		Gln	Ala	Ala	Lys 116		/ Lys		r Ile			e Phe	e Asp
Cys	Val		Thr	Ser	Thr	Lys 117		Thi	Gly	/ Ser	Thr	Glr	n Asr	ı Val	Tyr
Glu 118	Thr		Asp	Ile	Asn 119	Lys		Gli	ı Ası		Asr		Туг	Thi	Gly
		Val	Phe		Ser		Leu	His				Ser	туг		1200 Pro
Cln	700	717	т1.	120		7.00	01.	. m.	121			-	~ 1	121	.5
			122	0				122	:5 ·				123	0	Thr
		1235	5				124	0				124	5		Ile
Met	Glu 1250		Gly		Val					Asn			Asn	Gly	Ala
Leu	Ala	Ile	Asn	Gly	Leu	Thr							Gly	Thr	Pro
126	5				127	0				127	5				1280
				128	5				129	0				129	Thr 5
Thr	Ser	Ser	Ala	Ser	Gly	Gly	Ser	Gly	Val	Ser	Ser	Ser	Ile	Pro	Thr
Asn	Pro	Lys	1300 Arg		Ser	Ala	Ala	130 Val		Ser	Gly	Ser	131 Ala		Thr
		1315	5				132	0				132	5		
Thr	Pro 1330		Met	Ser	Glu	Asn 133		Val	Phe	Leu	Thr 134		Asp	Leu	Thr
Leu	Ile	Asp	Pro	Asn	Gly	Asn	Phe	Tyr	Gln	Asn	Pro	Met	Leu	Gly	Ser
1345					1350					135					1360
Asp	Leu	Asp	Val			Ile	Lys	Leu			Asn	Thr	Ser	Asp	Va:
(°1 n	1701	Th	7	1369			0	a i	137		_,	_		137	
GIII	Val	Tyr	1380		Thr	ьeu	ser			ьеи		Pro	GIn 139		Gly
Tyr	Met	Gly			Thr	Leu	Asp	Ser	Asn	Pro	Gln	Thr			Len
		1395				•	1400)				140	5		
Gln	Ala	Arg	Trp	Thr	Phe	Asp	Thr	Tyr	Arg	Arg	Trp	Val	Tyr	Ile	Pro
	1410					1415	5				1420)			
1425					1430)				1439	5				1440
Met	Ile	Val	Val	Lys 1445		Gly	Leu	Ile	Asn 1450		Met	Leu	Asn	Asn 1455	
Arg	Phe	Asp.	Asp	Ile	Ala	Tyr	Asn	Asn			Val	Ser	Glv	Val	Glv
			1460					1465	5				1470)	
Thr	Phe	Leu . 1475	Ala	Gln	Gln	Gly	Thr 1480		Leu	Ser	Glu	Glu 1485		Ser	Tyr
ľyr	Ser 1490	Arg	Gly	Thr	Ser	Val 1495		Ile	Asp	Ala	Lys 1500	Pro		Gln	Asp
	Ile		Gly	Ala	Ala			Lvs	Ile	Val			Thr	Lvs	Δla
1505			-		1510			•	_	1515		-1-		-,, -	1520
le	Lys :	Lys !		His 1525		Tyr	Phe	His	Lys 1530		Ser	Glu	Tyr	Ser 1535	Tyr
in .	Ala			Tyr		Gly			Leu		Phe	Leu		Asn	
3ln	His	Gly :	rp .		Leu		Phe			Gln					Tyr
:152	His :	1555 _. 11e i		ui e	Δαη		1560 Th∽		T 0	Тъ г∽		1565		112 =	01
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465					470					475				-	His 480
				485					490					495	Ser
			500					505					510		Asp
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	530					535					540		Trp		
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625					630					635			Ser		640
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			660					665					His 670		
		675					680					685	Ala		
ser	ьeu	ьys	Asn	ser	Ala	Glu	ьeu	Thr	Pro	Ser	Gly	His	Pro	Phe	Trp

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 Arg

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 Val
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 Ile
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 Asp
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 Gly
 Arg
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 Glu
 Phe
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 Asp

 Asp
 Val
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 Ala
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 Arg
 Leu
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 Val
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 Thr
 Val
 Glu
 Lys
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 Val
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 Asp
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llahlahah dan minik gilagi figarik saga seperara sa sa sa sa sa sa sa sa ka sa sa sa sa sa minin in ka ka sa ka

				85					90					95	
			100	١				105	;				110)	e Ala
Lys	Arg	y Val 115		Ile	Val	Asp	120		Glı	ı Ala	a Val	l Val		e Sei	Asn
Asn	Phe 130		Asp	Ile	Tyr	Gly 135		Ala	Ile	Phe	Thr 140		/ Ser	Lei	ı Arg
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				165					170)				175	
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225					230					235					Ala 240
				245					250					255	Thr
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		Asp		405					410				_	415	
		Pro	420					425					430		=
		Asn 435					440					445			
	450	Leu				455					460				
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		Gln		485					490					495	_
		Met	500					505					510		
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Ala-Leu Lys Asn Ala Arg Phe Ala His Asn Leu Thr Ala Gln Arg Met
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Glu Phe Asp Tyr Ser Thr Asn Val Trp Gly Phe Ala Phe Gly Gly Phe
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Ala Tyr Gly Gly Ala Ser Ala Gly Val Asp Ile Gln Leu Met Glu Asp
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Phe Val Leu Gly Val Ser Gly Ala Ala Phe Leu Gly Lys Met Asp Ser
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Glu Ser Ser Ala Ser Trp Thr Ser Arg Gly Val Leu Ala Asp Ala Leu
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Glu Leu Arg Val Ala Leu Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe
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Val Leu Leu Asp Gln Ile Arg Asp Leu Phe Val Gly Ser Lys Asp
                                              45
                           40
Ser Gln Ala Glu Gly Gln Tyr Arg Leu Ile Val Gly Asp Pro Ser Ser
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Phe	Glr	ı Glu	ı Lys	s Asp	70	a Asp	Thi	: Let	ı Pro	Gl _y 75	/ Lys	s Val	l Glu	ı Glı	ser 80
Thr	Leu	ı Phe	Ser	Va]	. Thr	Asr	Pro	Val	l Val 90	l Phe	e Glr	ı Gly	/ Val	L_Asp 95	Gln
Glr	Asp	Glr	100		Ser	Glr	ı Gly	Let 105		e Cys	Ser	Phe	Thr 110		Ser
Asn	Leu	115		Pro	Arg	Asp	Gly 120		ser Ser	Phe	e Leu	ı Gly 125		e Ala	Phe
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			180					185					190		Gly
		195					200					205	_		Ser
	210					215					220				Gly
225					230					235					Val 240
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	290				Ser	295					300				
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		355			Asn		360					365	_		
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	450				Gln	455					460				
465					Ala 470					475					480
Lys	Thr	Phe	Ala	Ser 485	Asn	Gly	Lys	Ile	Leu 490	Gly	Gly	Gly	Ala	Ile 495	Leu
Ala	Thr	Gly	Lys	Val	Glu	Ile	Thr	Asn	Asn	Ser	Gly	Gly	Ile	Ser	Phe

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Phe Ser Lys Asn Ser Ala Thr Asn Asn Ala Asn Asn Ala Thr Asp Thr

~1	_	_	100					105					110		
		115					120)				125	5	-	
Ser	Leu 130		Gly	Gly	/ Ala	His 135		Leu	Glu	i Asr	val 140		a Asp	Leu	ı Gly
Ser 145		Ile	Gly	Leu	Val 150		Asp	Thr	Glr	155		Glu	Thr	· Val	Lys 160
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Ala	Ile	Arg	Thr	Ser 325		Lys	Lys	Thr	Gly 330		Gln	Ala	Ťhr	Ala 335	Tyr
Asp	Thr	Leu	Asp 340	Ile	Asn	Lys	Ser	Glu 345		Ser	Glu	Thr	Val 350		
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Ser	Tyr 370	Ile	Pro	Gln	Asn	Val 375	Val	Leu	His	Ser	Gly 380		Leu	Val	Leu
Lys 385	Pro	Asn	Thr	Glu	Leu 390	His	Val	Ile	Ser	Phe 395		Gln	Lys	Glu	Gly 400
Ser	Ser	Leu	Val	Met 405	Thr	Pro	Gly	Ser	Val 410		Ser	Asn	Gln	Thr 415	Val
Ala	Asp	Gly	Ala 420	Leu	Val	Ile	Asn	Asn 425		Thr	Ile	Asp	Leu 430		Ser
Val	Glu	Lys 435	Asn	Gly	Ile	Ala	Glu 440		Asn	Ile	Phe	Thr 445		Pro	Glu
Leu	Arg 450	Ile	Ile	Asp	Thr	Thr 455	_	Ser	Gly	Ser	Gly 460		Thr	Pro	Ser
Thr 465	Asp	Ser	Glu	Ser	Asn 470		Asn	Ser	Asp	Asp 475		Lys	Glu	Gln	Asn 480
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Pro	Ala	Val	Ala 500		Ala	His	Thr	Ser 505		Thr	Arg	Asn	Phe 510		Ala
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Ser	Asn 530		Val	Ile	Leu	Gly 535		Glu	Ile	Lys	Leu 540		Asp	Pro	Asn
	-										-				

															•
Glv	Thr	Phe	Phe	Gln	Asn	Pro	Ala	Leu	Arg	Ser	Asp	Gln	Gln	Ile	Ser
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Leu	Leu	Val	Leu	Pro	Thr	Asp	Ser	Ser	Lys	Met	Gln	Ala	Gln	Lys	Ile
				565					570					5/5	
Val	Leu	Thr		Asp	Ile	Ala	Pro		Lys	GIY	Tyr	Tnr	Gly 590	THE	ьeu
	_	_	580		01	T	<i>(</i> 15	585	Clv	Thr	Tle	Ser	Ala	Leu	Trp
Thr	Leu	Asp 595	Pro	Asp	GIN	Leu	600	ASII	GIY	1111	110	605	1110		
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Phe	Tyr	Ala	Asn	Ser	Ile	Leu	Gly	Ser	Gln	Met	Ser	Met	Val	Thr	Val
625					630					635					64 U
Lys	Gln	Gly	Leu		Asn	Asp	Lys	Met	Asn	Leu	Ala	Arg	Phe	Asp 655	Glu
			_	645			T 1 -	Com	650	Ton	Clv	Thr	Met		Ser
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Cln	Val	Gly	660 Thr	Pro	Thr	Ser	Glu		Phe	Thr	Tyr	Tyr	Ser	Arg	Gly
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705		_	_,		710	71	0	C1.,	Th ex-	715	ባኒም	Gln	Δla	Ser	
Asn	Asn	Tyr	Thr	H1S	ьуѕ	GIY	Ser	GIU	730	261	ıyı	0111	Ala	735	
Tree	Gly	Glv	LMS	Pro	Phe	His	Phe	Val		Asn	Lys	Lys	Thr	Glu	Lys
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His	Asp	Thr	Val	Thr	His		Pro	Thr	Ile	Arg	Glu	Arg	Asn	GIN	GIY
~-	770	~ 1	•	T	a1	775	T 011	Thr	λla	T.em	780	Val	Ser	Ser	Val
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785	Ara	Thr	Pro	Ala	Gln	Glv	Asp	Thr	Lys		Ile	Thr	Val	Tyr	Gľy
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Tyr	Asp			Tyr	Phe	Asp			Thr	Tyr	Arg	845	Leu	MIG	116
D-+-		835		חות	Dhe	Glu	840	Glu	Leu	Ser	Glv		Asp	Ile	Leu
Pro	850		ьeu	нта	PIIC	855		014	cu	001	860				
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865					870					875					880
Ser	Pro	Thr	Cys	Lys	Tyr	Gln	Val	Leu	Ser	Ser	Gly	Glu	Gly	Gly	Glu
				885					890					895	
Ile	Ile	Суз			Pro	Thr	Arg			Ala	Arg	GIY	910	ıyı	Ser
	~1		900	Dec	. Cl.	. Dro	Tou	905		T.eu	Tyr	Glv			Thr
Thr	GIR	ьеч 915		PIO	о сту	PIC	920		1111	neu	- y -	925	20-	-1	
Tle	Gli	A16	Asn	Ala	His	Thr			His	Met	Met	Asn	Cys	Gly	Ala
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				=					410					41E	
T	Com	T 0	Cor	405	T OU	Luc	Thr	U = 1	410 Thr	Leu	Thr	Ĺys	Δen	415 Ser	Ala
ьeu	ser	Leu						425	TIIL	пси	IIII	цуз	430	501	niu
Lvs	Glu	Ser	Glv	GÎV	Ala	lle			Asp	Leu	Ala	Ser		Pro	Thr
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	Pro	Glu	Val	Val		Ser	Ala	Lys			Arg	Phe	Phe	Ala	Ser
465		~1			470	D	0	T 0	The	475	א ז ה	Clu	Cor		480
Thr	Ala	GIU	Pro	A1a 485	Ala	Pro	ser	Leu	490	GIU	АІА	Glu	ser	495	GIII
Thr	λen	Gln	Thr		Thr	Ser	Asp	Thr		Ser	Asp	Ile	Asp		Ser
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Lys		Gly	Ala	Ile	Tyr		Lys	Lys	Ala	Lys		Ser	Arg	Ile	Asn
_	530	~ 3	.	<i>-</i>	01	535	G = 10	C	C1-	7	540	C111	Clv	Clv	Lou
Asn 545	ьеu	GIU	Leu	Ser	550	ASII	Ser	Ser	GIII	555	vai	Gly	GIY	Gry	560
	Leu	Thr	Glu	Ser		Glu	Phe	Asp	Ala		Gly	Ser	Leu	Leu	
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His	Tyr	Asn	Ser	Ala	Ala	Lys	Glu	Gly	Gly	Val	Ile	His		Lys	Thr
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vai	610	Ald	116	vaı	GLu	615	1111	FIO	Giu	AIU	620	014	014		
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Thr	Glu	Gly	Ser	Ser	Ala	Asn	Thr	Asn		Glu	Gly	Ser	Gln		Asp
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Thr	Ala	Asp		Gly	Thr	Gly	Val	Val 665	Asn	Asn	GIU	Ser	670	Asp	IIII
Sar	λαη	Thr	660	Δan	Δla	Glui	Ser		Glu	Gln	Leu	Gln		Ser	Thr
361	Азр	675	Oly	71511	ALG	014	680	017		0		685			
Gln	Ser		Glu	Glu	Asn	Thr	Leu	Pro	Asn	Ser	Ser	Ile	Asp	Gln	Ser
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705	61	0	*** 1	0	710	C = 20	Com	T	202	715	Cor	Sor	Thr	Dro	720 Gln
Asp	G1u	Ser	vai	725	ser	ser	ser	ьуѕ	730	GIĀ	ser	Ser	1111	735	GIII
Asp	Glv	Glv	Ala		Ser	Ser	Glv	Ala		Ser	Gly	Asp	Gln		Ile
	017		740									-			
Ser	Ala	Asn	Ala	Cys	Leu	Ala	Lys	Ser	Tyr	Ala	Ala	Ser	Thr	Asp	Ser
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3	770		0	0	C	775	C1	7.00	Cor	ת 1 ת	780	λαη	Sor	Glu	Glv
785	rro	Asp	ser	ser	5er	ser	GIĀ	Asp	261	795	СТУ	Asp	UCI	CIU	800
	Thr	Glu	Pro	Glu		Glv	Ser	Thr	Thr		Thr	Pro	Thr	Leu	
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Gly	Gly	Gly	Ala	Ile											
			820												

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Trp Gln Val Gly Leu Ala Leu Ser Tyr Arg Leu Asn Met Leu Val Pro
                                    410
                405
Tyr Ile Gly Val Asn Trp Ser Arg Ala Thr Phe Asp Ala Asp Thr Ile
                                                    430
                                425
            420
Arg Ile Ala Gln Pro Lys Leu Lys Ser Glu Ile Leu Asn Ile Thr Thr
                            440
Trp Asn Pro Ser Leu Ile Gly Ser Thr Thr Ala Leu Pro Asn Asn Ser
                                            460
                        455
Gly Lys Asp Val Leu Ser Asp Val Leu Gln Ile Ala Ser Ile Gln Ile
                                        475
                   470
Asn Lys Met Lys Ser Arg Lys Ala Cys Gly Val Ala Val Gly Ala Thr
                                    490
                485
Leu Ile Asp Ala Asp Lys Trp Ser Ile Thr Gly Glu Ala Arg Leu Ile
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Asn Glu Arg Ala Ala His Met Asn Ala Gln Phe Arg Phe
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 gcaatc
 <210> 200
 <211> 34
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caatc	
•	_
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12137 Childhiyula	
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	30
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400 006	
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•	
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<211> 40	•
2212> DNA	
213> Chlamydia	

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<210> 20 <211> 50 <212> DI <213> CI	5	20 II 1			
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cagageta	agc atgcatcacc	atcaccatca	cgttaagatt	gagaacttct ctggc	55
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	atg catcaccatc	accatcacgg	gttagc		36
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  <211> 31
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  <213> Chlamydia
  <400> 216
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                                                                          31
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  tgcaatc
  <210> 218
  <211> 22
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                                                                         51
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 <211> 33
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Val Pro His His His His His
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                                                                         46
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Lys Asn Ser Ala Phe Met His Pro Val Asn Ile Ser Thr Asp Leu Ala
Val Ile Val Gly
            20
<210> 226
<211> 20
<212> PRT
<213> Artificial Sequence
<220>
<223> Made in a lab
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<400> 226
 His Pro Val Asn Ile Ser Thr Asp Leu Ala Val Ile Val Gly Lys Gly
      . . . .
 Pro Met Pro Arg
             20
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 <211> 20
 <212> PRT
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 <223> Made in a lab
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Ser Thr Asp Leu Ala Val Ile Val Gly Lys Gly Pro Met Pro Arg Thr
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Glu Ile Val Lys
<210> 228
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Val Ile Val Gly Lys Gly Pro Met Pro Arg Thr Glu Ile Val Lys Lys
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Val Trp Glu Tyr
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Gly Pro Met Pro Arg Thr Glu Ile Val Lys Lys Val Trp Glu Tyr Ile
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Lys Lys His Asn
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Ile Lys Lys His Asn Cys Gln Asp Gln Lys Asn Lys Arg Asn Ile-Leu
                                    10
Pro Asp Ala Asn
            20
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<211> 20
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Asn Cys Gln Asp Gln Lys Asn Lys Arg Asn Ile Leu Pro Asp Ala Asn
Leu Ala Lys Val
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Lys Asn Lys Arg Asn Ile Leu Pro Asp Ala Asn Leu Ala Lys Val Phe
                                    10
Gly Ser Ser Asp
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Ile Leu Pro Asp Ala Asn Leu Ala Lys Val Phe Gly Ser Ser Asp Pro
1
Ile Asp Met Phe
<210> 234
<211> 20
<212> PRT
<213> Artificial Sequence
<220>
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<223> Made in a lab
_<400> 234 _ _ _
 Asn Leu Ala Lys Val Phe Gly Ser Ser Asp Pro Ile Asp Met Phe Gln
 Met Thr Lys Ala
             20
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 <211> 22
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Ser Lys His Ile Val Lys
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Tyr Pro Val Glu
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Thr Ala Thr Gly
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<210> 238
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<220>
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 Ala Thr Val Gly Ser Pro Tyr Pro Val Glu Ile Thr Ala Thr Gly Lys
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 Arg Asp Cys Val
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 Pro Tyr Pro Val Glu Ile Thr Ala Thr Gly Lys Arg Asp Cys Val Asp
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Val Ile Ile Thr
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Gln Leu Pro Cys Glu
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Ala Glu Phe Val
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 Asp Val Ile Ile Thr Gln Gln Leu Pro Cys Glu Ala Glu Phe Val Arg
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 Ser Asp Pro Ala
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 Thr Thr Pro Thr
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Asp Gly Lys Leu
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Trp Lys Ile Asp
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<213> Artificial Sequence
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Leu Gly Gln Gly
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Ala Asp Gly Lys Leu Val Trp Lys Ile Asp Arg Leu Gly Gln Gly Glu
Lys Ser Lys Ile
           20
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Val Trp Lys Ile Asp Arg Leu Gly Gln Gly Glu Lys Ser Lys Ile Thr
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Val Trp Val Lys
            20
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<400> 249
Arg Leu Gly Gln Gly Glu Lys Ser Lys Ile Thr Val Trp Val Lys Pro
1
Leu Lys Glu Gly
           20
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<211> 20
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<212> PRT
 <213> Artificial Sequence
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 <223> Made in a lab
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 Gly Glu Lys Ser Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly
 Cys Cys Phe Thr
             20
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Gly Glu Lys Ser Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly
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                                     10
<210> 252
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<223> Made in a lab
<400> 252
Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly
                 5
<210> 253
<211> 16
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<223> Made in a lab
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Gly Asp Lys Cys Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly
                                    10
<210> 254
<211> 20
<212> PRT
<213> Artificial Sequence
<220>
<223> Made in a lab
```

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<400> 254
Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser Phe Lys Ile Ser Glu Ala
1
                                    10
Phe Gly Val Leu
            20
<210> 255
<211> 20
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<223> Made in a lab
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1
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Pro Glu Gly Ser
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<223> Made in a lab
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Phe Lys Ile Ser Glu Ala Phe Gly Val Leu Asn Pro Glu Gly Ser Leu
                                    10
Ala Leu Arg Ala
            20
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<211> 20
<212> PRT
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Ala Phe Gly Val Leu Asn Pro Glu Gly Ser Leu Ala Leu Arg Ala Thr
Phe Leu Ile Asp
<210> 258
<211> 20
<212> PRT
<213> Artificial Sequence
<220>
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માં ભૂતિએ કર્યા તુમાર લેક્સ વસ્તર લેક્સ વેસ્ટર લેક્સ વારા મારા કર્યા છે. જે જ

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<223> Made in a lab
<400> 258
Asn Pro Glu Gly Ser Leu Ala Leu Arg Ala Thr Phe Leu Ile Asp Lys
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His Gly Val Ile
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<211> 20
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<400> 259
Leu Ala Leu Arg Ala Thr Phe Leu Ile Asp Lys His Gly Val Ile Arg
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                                     10
His Ala Val Ile
            20
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<211> 20
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<213> Artificial Sequence
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<223> Made in a lab
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Thr Phe Leu Ile Asp Lys His Gly Val Ile Arg His Ala Val Ile Asn
Asp Leu Pro Leu
            20
<210> 261
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<212> PRT
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Lys His Gly Val Ile Arg His Ala Val Ile Asn Asp Leu Pro Leu Gly
Arg Ser Ile Asp
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<210> 262
<211> 20
<212> PRT
<213> Artificial Sequence
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<220>
 <223> Made in a lab
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Arg His Ala Val Ile Asn Asp Leu Pro Leu Gly Arg Ser Ile Asp Glu
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Glu Leu Arg Ile
             20
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<220>
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attaaggttg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc
                                                                        180
gcgggctctt ccgcacacat tacagcttcc caagtgtcca aaggattagg ggatgcgaga
                                                                        240
actgttgtcg ctttagggaa tgcctttaac ggagcgttgc caggaacagt tcaaagtgcg
                                                                        300
caaagettet teteteacat gaaagetget agteagaaaa egeaagaagg ggatgagggg
                                                                        360
ctcacagcag atctttgtgt gtctcataag cgcagagcgg ctgcggctgt ctgtagcatc
                                                                        420
atcggaggaa ttacctacct cgcgacattc ggagctatcc gtccgattct gtttgtcaac
                                                                        480
aaaatgctgg caaaaccgtt tctttcttcc caaactaaag caaatatggg atcttctgtt
                                                                        540
agctatatta tggcggctaa ccatgcagcg tctgtggtgg gtgctggact cgctatcagt
                                                                        600
gcgnaaagag cagattgcga agcccgctgc gctcgtattg cgagagaaga gtcgttactc
                                                                        660 -
gaagtgccgg gagaggaaaa tgcttgcgag aagaaagtcg ctggagagaa agccaagacg
                                                                        720
ttcacgcgca tcaagtatgc actcctcact atgctcgaga agtttttgga atgcgttgcc
                                                                        780
gacgttttca aattggtgcc gctgcctatt acaatgggta ttcgtgcgat tgtggctgct
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ggatgtacgt teacttetge aattattgga ttgtgeactt tetgegeeag ageataa
                                                                        897
<210> 264
<211> 298
<212> PRT
<213> Chlamydia
<220>
<221> VARIANT
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<400> 264
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Lys Ala Phe Phe Thr Gln Pro Asn Asn Lys Met Ala Arg Val Val Asn
            20
                                 25
                                                     30
Lys Thr Lys Gly Val Asp Lys Thr Ile Lys Val Ala Lys Ser Ala Ala
                            40
                                                 45
Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
    50
```

Halled SAM aliktrillatur kaisal sejil Alal Albahahib shirur am manan mana mana mana mana kalisa katin pertimbal mana bilatur ar bar ar alaba ar arisa.

```
Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg
                     70
 Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr
                                      90
 Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln
                                  105
 Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser
                             120
                                                  125
 His Lys Arg Arg Ala Ala Ala Val Cys Ser Ile Ile Gly Gly Ile
                         135
                                              140
 Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
                                         155
 Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
                 165
                                     170
 Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
             18C
                                 185
 Val Gly Ala Gly Leu Ala Ile Ser Ala Xaa Arg Ala Asp Cys Glu Ala
         195
                             200
                                                 205
Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Pro Gly
                         215
                                             220
Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr
                     230
                                         235
Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
                                     250
Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
             260
                                 265
Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile
        275
                             280
                                                 285
Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala
    290
                         295
<210> 265
<211> 897
<212> DNA
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<221> misc_feature
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                                                                       120
attaaggttg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc
                                                                       180
gcgggctctt ccgcacacat tacagcttcc caagtgtcca aaggattagg ggatgcgaga
                                                                       240
actgttgtcg ctttagggaa tgcctttaac ggagcgttgc caggaacagt tcaaagtgcg
                                                                       300
caaagcttct tctctcacat gaaagctgct agtcagaaaa cgcaagaagg ggatgagggg
                                                                       360
ctcacagcag atctttgtgt gtctcataag cgcagagcgg ctgcggctgt ctgtagcatc
                                                                       420
atcggaggaa ttacctacct cgcgacattc ggagctatcc gtccgattct gtttgtcaac
                                                                       480
aaaatgctgg caaaaccgtt tctttcttcc caaactaaag caaatatggg atcttctgtt
                                                                       540
agctatatta tggcggctaa ccatgcagcg tctgtggtgg gtgctggact cgctatcagt
                                                                       600
gcgnaaagag cagattgcga agcccgctgc gctcgtattg cgagagaaga gtcgttactc
                                                                       660
gaagtgccgg gagaggaaaa tgcttgcgag aagaaagtcg ctggagagaa agccaagacg
                                                                       720
ttcacgcgca tcaagtatgc actcctcact atgctcgaga agtttttgga atgcgttgcc
                                                                       780
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897

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                                                                         120
 gttccttacg ttcagagaag gattttgtcg cgttagttgg taaagtttta gctgataacg
                                                                         180
 tagttgatgc ggattettea ttagtttaeg ggaaagetgg agagaageta agtaetgeta
                                                                         240
 tgctaaaacg catcttagat acgggagtcc aatctttgaa gattgctgtt ggcgcagatg
                                                                         300
 aaaatcaccc aattattaag atgctcgcaa aagatcctac ggattcttac gaagctgctc
                                                                         360
 ttaaaagattt ttatcgcaga ttacgaccag gagagcctgc aactttagct aatgctcgat
                                                                         420
 ccacaattat gcgtttattc ttcgatgcta aacgttataa tttaggccgc gttggacgtt
                                                                         480
 ataaattaaa taaaaaatta ggcttcccat tagacgacga aacattatct caagtgactt
                                                                        540
 tgagaaaaga agatgttatc ggcgcgttga aatatttgat tcgtttgcga atgggcgatg
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aactaattca gaatcactgt
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<211> 359
<212> DNA
<213> Chlamydia
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agetectaac aaagagetaa tttttteete tteettgttt ttetgaggeg etgtggaete
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taaatatago aagtgotott ggaacacoto atcaacaato gettgtoota gattaggtat
                                                                        240
agagactgtc totocatoaa ttaaatggag tttoaaagta atatoooott cogtocotoo
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ggtt
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<211> 219
<212> DNA
<213> Chlamydia
<400> 270
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cgctcttgtc caatgacata agagtcgatg tggcgtttga tttctttagg ggttaacact
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kalsallillukhillillukharistekikakitalen kisten k

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cctgttctcc atagatagct cctcctacta cacctgaata agttggtgtt gctggagatg
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atggtgcggc tgctgcggct gcttgtaggg aagcagcagc tgcagcaggt gctgaagctg
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ttgttgcgac tcctgtggat gaggagtttg ctttgttgtt cgagaaagag aagcctgatt
                                                                        360
tcagattaga aatatttaca gttttagcat gtaagcctcc accttettte ccaacaaggt
                                                                        420
                                                                        480
tctctgttac agataaggag actagangca tctagtttta aagatttttt acagcagata
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<400> 272
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ttatactgat aagaatcttt cgattactaa catcacagga attatcgaaa ttgcaaataa
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tcaccgtcta caatttttga aaaactcttc cgataaacaa ggtggaggaa tctacggaga
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                                                                        360
agacaacatc accctatcta atttgacagg gaagactcta ttccaagaga atactgccaa
aaaagagggc ggtggactct tcataaaagg tacagataaa gctcttacaa tgacaggact
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ggatagtttc tgtttaatta ataacacatc agaaaaacat ggtggtggga gcctttgtta
                                                                        480
ccaaagaaat ctctcagact tacacctctt gatgtggaaa caattccagg aatcacgcct
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<211> 126
<212> DNA
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cgagag
<210> 274
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<212> DNA
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ataaccatag ttacggggga atctctttca tggtttattt tagagctcat caacctaggc
                                                                        180
atacgcctaa aacatttcct ttgaaagttc accattcgtt ctccgataag catcctcaaa
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 ttaaaacttg ttctcttaaa ttaattctag tatttaagta ttcaacatag cccattatta
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 attgaattgg ataattttgc cttaataatt cacattcttt ttcagtaatt ttaggttcta
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 aaccgtaccg ctttttttct aaaattaatg tttcttcatt attcatttta taagccactt
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 <211> 357
 <212> DNA
 <213> Chlamydia
<400> 276
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tgatgaaaac ggaaacatcc tttcgccaga aactttagca ctattaaaga atcgttacgg
                                                                        180
gttagataag cetttattea eccagtater tatetatttg aaatgtetge taacactaga
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tttcggggaa tctcttatct acaaagatcg aaatctcagc attattgctg ccgctcttcc
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atcttccgct attcttggac ttgaaagctt gtgtttactc gtgccgaatt cggatcc
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<212> DNA
<213> Chlamydia
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ggtaaaaatc ctaaggccat accaggatgc gacaggaaag agatatctcc attaggagct
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cggagacacg ctgggttgtg gccacaagaa tagtattcta gttctcgtgt tgcgtaatga
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taacaataaa tgcatagtgt tacaaacatc ccagattcag ctgtctgttg atagaagaga
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gcagctgttt gttgaacggc ttcttgaata gaggagagct cactcaaaaa ggtatgtaac
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atgtttttca ggaataagga gtaggcgcac gcattgactc ctttcccgga agcatcagca
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acgattagaa agagtttagc ttggggacct tcgcctataa caaagatatc aaagaaatct
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                                                                       120
ctttggctct gctaactgga gcggtgctgg tatgattaaa aactttgaag acctattcat
                                                                       180
ccttcgccca attacagaga cacagcttca ggcctttatg gacgtctggt ctcttctaga
                                                                       240
aacaaatage teetatetgt eeceagagag egtgettaeg geecetaete etteaagtag
                                                                       300
acctactcaa caagatacag attctgatga cgaacaaccg agtaccagcc agcaagctat
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131

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                                                                        120
ggatcgtata ctttttcaaa gtatggtccc cgtatcgatt atctggaggc tcttatgtct
                                                                        180
ttttttcata ctagaaaata taagcttatc ctcagaggac tcttgtgttt agcaggctgt
                                                                        240
ttottaatga acagetgtto ototagtoga ggaaatcaac coqotqatqa qaqoatctat
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tgattettet tetgaegaaa ttetegatge geteacaagt aaattttetg ateccacaat
                                                                       180
aaaggatota gotottgatt atotaattoa aatagotooo totgatggga aacttaagto
                                                                       240
egeteteatt caggeaaage ateaactgat gagecagaat eeteaggega ttgttggagg
                                                                       300
acgcaatgtt ctgttagctt cagaaacctt tgcttccaga gcaaatacat ctccttcatc
                                                                       360
gettegetee ttatatttee aagtaacete atceeeetet aattgegeta atttacatea
                                                                       420
aatgettget tettaetege cateagagaa aacegetgtt atggagttte tagtgaatgg
                                                                       480
catggtagca gatttaaaat cggagggccc ttccattcct cc
                                                                       522
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<211> 577
<212> DNA
<213> Chlamydia
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Ccagcttatt ctagaaaagt tgggagatca aattcttggt ggaattgctq atactattqt
                                                                       120
tgatagtaca gtccaagata ttttagacaa aatcacaaca gacccttctc taggtttgtt
                                                                       180
gaaagctttt aacaactttc caatcactaa taaaattcaa tgcaacgggt tattcactcc
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caggaacatt gaaactttat taggaggaac tgaaatagga aaattcacag tcacacccaa
                                                                       300
aagctctggg agcatgttct tagtctcagc agatattatt gcatcaagaa tggaaggcgg
                                                                       360
cgttgttcta gctttggtac gagaaggtga ttctaagccc tacgcgatta gttatggata
                                                                       420
ctcatcagge gttectaatt tatgtagtet aagaaccaga attattaata caggattgae
                                                                       480
tecgacaacg tatteattae gtgtaggegg tttagaaage ggtgtggtat gggttaatge
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cctttctaat ggcaatgata ttttaggaat aacaaat
                                                                       577
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<211> 607
<212> DNA
<213> Chlamydia
<400> 282
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<213> Chlamydia

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                                                                        180
gctgaaaaaa cctaaattca aaagaatgac tcgccgctca tcttcagaaa gacgatccga
                                                                       - 240-
cttccataat tcgatgtctt tccccatggg gatctctgta gggagccagt tatttgcgca
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gccattcaaa taatgttccc aagcccattt gtacttaata ggaacaagtt ggttgacatc
                                                                        360
gacctggttg cagttcacta gacgcttgct atttagatta acgcgtttct gttttccatc
                                                                        420
taaaatatct gcttgcataa gaaccgttaa ttttattgtt aatttatatg attaattact
                                                                        480
gacatgcttc acacccttct tccaaagaac agacaggtgc tttcttcgct ctttcaacaa
                                                                        540
taatteetge egaageagae ttattettea teeaaegagg etgaatteet etettattaa
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<211> 1077
<212> DNA
<213> Chlamydia
<400> 283
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ttctagtaag caggaaaaaa gctcgtaacg cctcttcatc ggtggctaat gtataaaagg
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ctcgtcctga ctcatgcatt tcggcatgat ctggcccaac tgaaggataa tctaatccaq
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cggaaatgga gtgagtttgt aatacttgtc catcgtcatc ttgaagaaga tacgaataaa
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atccgtggaa tactccaggt cgccctgttg caaaacgtgc tgcatgtttt cctgaagaaa
                                                                       360
tgcccagtcc tcccccttcc actccaatta attggacttt tggattcggg ataaaatgat
                                                                       420
ggaaaaatcc aatagcgttg gagccacctc cgatacatgc aatcagaata tcaggatctc
                                                                       480
ttcctgcaac tgcatggatt tgctctttca cttcagcgct tataacagac tgaaaaaatc
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gaacgatatc gggataaggt aaaggtccta aggccgatcc taagcaatag tgagtaaatq
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agtgtgttgt tgcccaatct tgtagagctt gattaactgc atctttgagt ccacaagatc
                                                                       660
cttttgttac agaaacgact tcagcaccta aaaagcgcat tttctctaca tttqqtttct
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gtcgttccac atcttttgct cccatgtata ctacacaatc taatcctaga taaqcacacq
                                                                       780
etgttgetgt tgetaeteea tgttgteeeg eacetgttte agetaeaaca egtgttttee
                                                                       840
caagatattt agcaagcaaa cactgaccaa gagcattatt cagtttatgt gctcctgtat
                                                                       900
gcaaaagatc ttcgcgttta agaaatactc tagggccatc aatagctcga gcaaaattct
                                                                       960
taacttcagt cagaggagtt tgtctccccg catagttttt caaaatacaa tctagttcag
                                                                      1020
ataaaaaact ttgctgagtt ttgagaatct cccattccgc ttttaqattc tqtataq
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<213> Chlamydia
<400> 284
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aagaaaaaca gaaggcattc tccataccaa gatttgttgc atcgacaata aaactccaat
                                                                       120
ctttggctct gctaactgga gcggtgctgg tatgattaaa aactttgaag acctattcat
                                                                       180
cettegecca attacagaga cacagettea ggeetttatg gaegtetggt etettetaga
                                                                       240
aacaaatagc tectatetgt eeccagagag egtgettaeg geecetaete etteaagtag
                                                                       300
acctactcaa caagatacag attctgatga cgaacaaccg agtaccagcc agcaagctat
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ccgtatgaga aaataggatt agggaaacaa aacgacagca aaccaca
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<211> 802
<212> DNA
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tgggaaaaaa gaatctgctg aattcgaaaa gatgaaaaac caattctcta acagcatggg
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gaagatggag gaagaactgt cttctatcta ttccaagctc caagacgacg attacatgga
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aggtctatcc gagaccgcag ctgccgaatt aagaaaaaaa ttcgaagatc tatctgcaga
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atacaacaca gctcaagggc agtattacca aatattaaac caaagtaatc tcaagcgcat
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gcaaaagatt atggaagaag tgaaaaaagc ttctgaaact gtgcgtattc aagaaggctt
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gtcagtcctt cttaacgaag atattgtctt atctatcgat agttcggcag ataaaaccga
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tgctgttatt aaagttcttg atgattcttt tcaaaataat taacatgcga agctagccga
                                                                        540
ggagtgccgt atgtctcaat ccacttattc tcttgaacaa ttagctgatt ttttgaaagt
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cgagtttcaa ggaaatggag ctactcttct ttccggagtt gaagagatcg aggaagcaaa
                                                                        660
aacggcacac atcacattct tagataatga aaaatatgct aaacatttaa aatcatcgga
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agctggcgct atcatcatat ctcgaacaca gtttcaaaaa tatcgagact tgaataaaaa
                                                                        780
ctttcttatc acttctgagt ct
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<212> DNA
<213> Chlamydia
<400> 286
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                                                                        120
attgcaaccg cacgcgattg aatgatacgc aagccatttc catcatggaa aagaaccctt
                                                                        180
ggacaaaaat acaaaggagg ttcactccta accagaaaaa gggagagtta gtttccatgg
                                                                        240
gttttcctta tatacacccg tttcacacaa ttaggagccg cgtctagtat ttggaataca
                                                                        300
aattgtcccc aagcgaattt tgttcctgtt tcagggattt ctcctaattg trctgtcagc
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catccgccta tggtaacgca attagctgta gtaggaagat caactccaaa caggtcatag
                                                                        420
aaatcagaaa getestaggt geetgeagea ataacaacat tettgtetga gtgagegaat
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tgtttaaaag atgggcgatt atgagctacc tcatcagaga ctattttaaa tagatcattt
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tgggtaatca atccttctat agacccatat tcatcaatga taatctcg
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\langle 223 \rangle n = A,T,C or G
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acaagtagct gttatgtatg gttctagttg cttactgcgc gccgtgggcg atttagcgaa
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aaatgattct tctattcaag tacgcatcac tgcttatcgt gctgcagccg tgttggagat
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acaagatett gtgeeteatt tacgagttgt agteeaaaat acacaattag atggaacgga
                                                                       240
aagaagagaa gettggagat etttatgtgt tettaetegg eeteatagtg gtgtattaae
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tggcatagat caagetttaa tgaeetgtga gatgttaaag gaatateetg aaaagtgtae
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ggaagaacag attcgtacat tattggctgc agatcatcca gaagtgcagg tagctacttt
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acagatcatt ctgagaggag gtagagtatt ccggtcatct tctataatgg aatcggttct
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cgtgccgnt
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<210> 288
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PCT/US99/29012 134

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agaaaaacgc ctggggagta tagtaaaatg ctattaactc gaggtgatta cctattggca
gettecaqqq aaqettqtac qqcaqtegqt gcaacqactt actcaqcqac attcqqtqtt
ttacgtccgt taatgttaat caataaactc acagcaaaac cattcttaga caaagcgact
                                                                      600
gtaggcaatt ttggcacggc tgttgctgga attatgacca ttaatcatat ggcaggagtt
                                                                      660
gctggtgctg ttggcggaat cgcattagaa caaaagctgt tcaaacgtgc gaaggaatcc
                                                                      720
ctatacaatg agagatgtgc cttagaaaac caacaatctc agttgagtgg ggacgtgatt
                                                                      780
                                                                      840
ctaagcgcgg aaagggcatt acgtaaagaa cacgttgcta ctctaaaaaag aaatgtttta
actettettg aaaaagettt agagttggta gtggatggag teaaaeteat teetttaeeg
                                                                      900
attacagtgg cttgctccgc tgcaatttct ggagccttga cggcagcatc cgcaggaatt
                                                                      960
ggcttatata gcatatggca gaaaacaaag tctggcaaat aa
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<211> 333
<212> PRT
<213> Chlamydia
<400> 292
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1
                5
                                    10
Leu Leu Pro Val Ala Lys Glu Pro Ala Ala Val Ser Ser Phe Ala Gln
           20
                                25
Lys Gly Ile Tyr Cys Ile Gln Gln Phe Phe Thr Asn Pro Gly Asn Lys
                           40
Leu Ala Lys Phe Val Gly Ala Thr Lys Ser Leu Asp Lys Cys Phe Lys
Leu Ser Lys Ala Val Ser Asp Cys Val Val Gly Ser Leu Glu Glu Ala
                   70
                                       75
Gly Cys Thr Gly Asp Ala Leu Thr Ser Ala Arg Asn Ala Gln Gly Met
               85
                                    90
Leu Lys Thr Thr Arg Glu Val Val Ala Leu Ala Asn Val Leu Asn Gly
           100
                               105
Ala Val Pro Ser Ile Val Asn Ser Thr Gln Arg Cys Tyr Gln Tyr Thr
       115
                           120
                                               125
Arg Gln Ala Phe Glu Leu Gly Ser Lys Thr Lys Glu Arg Lys Thr Pro
                       135
                                           140
Gly Glu Tyr Ser Lys Met Leu Leu Thr Arg Gly Asp Tyr Leu Leu Ala
                   150
                                        155
Ala Ser Arg Glu Ala Cys Thr Ala Val Gly Ala Thr Thr Tyr Ser Ala
               165
                                    170
Thr Phe Gly Val Leu Arg Pro Leu Met Leu Ile Asn Lys Leu Thr Ala
           180
                               185
Lys Pro Phe Leu Asp Lys Ala Thr Val Gly Asn Phe Gly Thr Ala Val
                           200
                                               205
Ala Gly Ile Met Thr Ile Asn His Met Ala Gly Val Ala Gly Ala Val
                                          220
                       215
Gly Gly Ile Ala Leu Glu Gln Lys Leu Phe Lys Arg Ala Lys Glu Ser
                   230
                                       235
Leu Tyr Asn Glu Arg Cys Ala Leu Glu Asn Gln Gln Ser Gln Leu Ser
               245
                                   250
Gly Asp Val Ile Leu Ser Ala Glu Arg Ala Leu Arg Lys Glu His Val
                               265
Ala Thr Leu Lys Arg Asn Val Leu Thr Leu Leu Glu Lys Ala Leu Glu
                           280
                                               285
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Leu Val Val Asp Gly Val Lys Leu Ile Pro Leu Pro Ile Thr Val Ala

વિકારિતારી વિકાશ પ્રત્યાન કરવા કરિકારિતારી કરિકારી વસ્તા કરવા હતા કરવા છે. કું તેમજ કરવા વસ્તા વસ્તા કરવા વાદ વ ત્યારા કરિકારિતા કું તેમાં કું તેમાં કું તેમાં કું તાલા કું તે તેમાં કું તે તેમાં કું તે તે જે તે કું તે તે કે

Cys Ser Ala Ala Ile Ser Gly Ala Leu Thr Ala Ala Ser Ala Gly Ile

305 310 315 Gly Leu Tyr Ser Ile Trp Gln Lys Thr Lys Ser Gly Lys 325 <210> 293 <211> 7 <212> DNA <213> Chlamydia <400> 293 tgcaatc <210> 294 <211> 196 <212> PRT <213> Chlamydia <400> 294 Thr Met Gly Ser Leu Val Gly Arg Gln Ala Pro Asp Phe Ser Gly Lys Ala Val Val Cys Gly Glu Glu Lys Glu Ile Ser Leu Ala Asp Phe Arg Gly Lys Tyr Val Val Leu Phe Phe Tyr Pro Lys Asp Phe Thr Tyr Val Cys Pro Thr Glu Leu His Ala Phe Gln Asp Arg Leu Val Asp Phe Glu Glu His Gly Ala Val Val Leu Gly Cys Ser Val Asp Asp Ile Glu Thr His Ser Arg Trp Leu Thr Val Ala Arg Asp Ala Gly Gly Ile Glu Gly 90 Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser Phe Lys Ile Ser Glu Ala 105 Phe Gly Val Leu Asn Pro Glu Gly Ser Leu Ala Leu Arg Ala Thr Phe 125 Leu Ile Asp Lys His Gly Val Ile Arg His Ala Val Ile Asn Asp Leu Pro Leu Gly Arg Ser Ile Asp Glu Glu Leu Arg Ile Leu Asp Ser Leu Ile Phe Phe Glu Asn His Gly Met Val Cys Pro Ala Asn Trp Arg Ser Gly Glu Arg Gly Met Val Pro Ser Glu Glu Gly Leu Lys Glu Tyr Phe

Gln Thr Met Asp 195

<210> 295

<211> 181

<212> PRT

<213> Chlamydia

<400> 295

Lys Gly Gly Lys Met Ser Thr Thr Ile Ser Gly Asp Ala Ser Ser Leu 5 10 15

Pro Leu Pro Thr Ala Ser Cys Val Glu Thr Lys Ser Thr Ser Ser Ser 26 25 30

Thr Lys Gly Asn Thr Cys Ser Lys Ile Leu Asp Ile Ala Leu Ala Ile 35 40 45

Val Gly Ala Leu Val Val Val Ala Gly Val Leu Ala Leu Val Leu Cys
50 60

Ala Ser Asn Val Ile Phe Thr Val Ile Gly Ile Pro Ala Leu Ile Ile 65 70 75 80

Gly Ser Ala Cys Val Gly Ala Gly Ile Ser Arg Leu Met Tyr Arg Ser 85 90 95

Ser Tyr Ala Ser Leu Glu Ala Lys Asn Val Leu Ala Glu Gln Arg Leu 100 105 110

Arg Asn Leu Ser Glu Glu Lys Asp Ala Leu Ala Ser Val Ser Phe Ile 115 120 125

Asn Lys Met Phe Leu Arg Gly Leu Thr Asp Asp Leu Gln Ala Leu Glu 130 135 140

Ala Lys Val Met Glu Phe Glu Ile Asp Cys Leu Asp Arg Leu Glu Lys 145 150 155 160

Asn Glu Gln Ala Leu Leu Ser Asp Val Arg Leu Val Leu Ser Ser Tyr
165 170 175

Thr Arg Trp Leu Asp 180

<210> 296

<211> 124

<212> PRT

<213> Chlamydia

<400> 296

Ile Tyr Glu Val Met Asn Met Asp Leu Glu Thr Arg Arg Ser Phe Ala

Skåltettelikollssikt itt kjeppera klegit meg år och er er er er er meg er fantakaknik kealik hedde om sker tahlin och er alatik kees

Val Gln Gln Gly His Tyr Gln Asp Pro Arg Ala Ser Asp Tyr Asp Leu 20 25 30

Pro Arg Ala Ser Asp Tyr Asp Leu Pro Arg Ser Pro Tyr Pro Thr Pro 35 40 45

Pro Leu Pro Ser Arg Tyr Gln Leu Gln Asn Met Asp Val Glu Ala Gly 50 60

Phe Arg Glu Ala Val Tyr Ala Ser Phe Val Ala Gly Met Tyr Asn Tyr 65 70 75 80

Val Val Thr Gln Pro Gln Glu Arg Ile Pro Asn Ser Gln Gln Val Glu 85 90 95

Gly Ile Leu Arg Asp Met Leu Thr Asn Gly Ser Gln Thr Phe Ser Asn 100 105 110

Leu Met Gln Arg Trp Asp Arg Glu Val Asp Arg Glu 115 120

<210> 297

<211> 488

<212> PRT

<213> Chlamydia

<400> 297

Phe Trp Arg Thr Ser Ile Met Lys Met Asn Arg Ile Trp Leu Leu Leu 20 25 30

Leu Thr Phe Ser Ser Ala Ile His Ser Pro Val Arg Gly Glu Ser Leu 35 40 45

Val Cys Lys Asn Ala Leu Gln Asp Leu Ser Phe Leu Glu His Leu Leu 50 55 60

Gln Val Lys Tyr Ala Pro Lys Thr Trp Lys Glu Gln Tyr Leu Gly Trp 65 70 75 80

Asp Leu Val Gln Ser Ser Val Ser Ala Gln Gln Lys Leu Arg Thr Gln 85 90 95

Glu Asn Pro Ser Thr Ser Phe Cys Gln Gln Val Leu Ala Asp Phe Ile 100 105 110

Gly Gly Leu Asn Asp Phe His Ala Gly Val Thr Phe Phe Ala Ile Glu 115 120 125

Ser Ala Tyr Leu Pro Tyr Thr Val Gln Lys Ser Ser Asp Gly Arg Phe 130 140

Tyr 145		Val	Asp	Ile	Met 150	Thr	Phe	Ser -	Ser	Glu 155	Ile	Arg	Val	Gly	Asp 160
Glu	Leu	Leu	Glu	Val 165	Asp	Gly	Ala	Pro	Val 170	Gln	Asp	Val	Leu	Ala 175	Thr
Leu	Tyr	Gly	Ser 180	Asn	His	Lys	Gly	Thr 185	Ala	Ala	Glu	Glu	Ser 190	Ala	Ala
Leu	Arg	Thr 195	Leu	Phe	Ser	Arg	Met 200	Ala	Ser	Leu	Gly	His 205	Lys	Val.	Pro
	210		Thr			215					220				
225			Val		230					235					240
			Ala	245					250					255	
			Phe 260					265					270		
		275	Ser				280					285			
	290					295					300				Thr
305	_		Leu		310					315					320
			Ala	325					330					335	
			340					345					350		Met
		355	Asp				360					365			
	370					375					380				Gln
Thr 385	Asn	Asn	Pro	Gly	Gly 390		Val	Leu	Tyr	Leu 395	Tyr	Ala	Leu	Leu	Ser 400
Met	Leu	Thr	Asp	Arg 405		Leu	Glu	Leu	Pro 410		His	Arg	Met	Ile 415	Leu
Thr	Gln	Asp	Glu 420		Val	Asp	Ala	Leu 425	Asp	Trp	Leu	Thr	Leu 430	Leu	Glu

Antillettellitatistalistaanista kan maran ma Talifatistalista maran mara

Asn Val Asp Thr Asn Val Glu Ser Arg Leu Ala Leu Gly Asp Asn Met
435
440
445

Glu Gly Tyr Thr Val Asp Leu Gln Val Ala Glu Tyr Leu Lys Ser Phe 450 460

Gly Arg Gln Val Leu Asn Cys Trp Ser Lys Gly Asp Ile Glu Leu Ser 465 470 475 480

Thr Pro Ile Pro Leu Phe Gly Phe 485

<210> 298

<211> 140

<212> PRT

<213> Chlamydia

<400> 298

Arg Ile Asp Ile Ser Ser Val Thr Phe Phe Ile Gly Ile Leu Leu Ala
5 10 15

Val Asn Ala Leu Thr Tyr Ser His Val Leu Arg Asp Leu Ser Val Ser 20 25 30

Met Asp Ala Leu Phe Ser Arg Asn Thr Leu Ala Val Leu Leu Gly Leu
35 40 45

Val Ser Ser Val Leu Asp Asn Val Pro Leu Val Ala Ala Thr Ile Gly
50 55 60

Met Tyr Asp Leu Pro Met Asn Asp Pro Leu Trp Lys Leu Ile Ala Tyr 65 70 75 80

Thr Ala Gly Thr Gly Gly Ser Ile Leu Ile Ile Gly Ser Ala Ala Gly 85 90 95

Val Ala Tyr Met Gly Met Glu Lys Val Ser Phe Gly Trp Tyr Val Lys
100 105 110

His Ala Ser Trp Ile Ala Leu Ala Ser Tyr Phe Gly Gly Leu Ala Val 115 120 125

Tyr Phe Leu Met Glu Asn Cys Val Asn Leu Phe Val 130 135 140

<210> 299

<211> 361

<212> PRT

<213> Chlamydia

<400> 299

His Gln Glu Ile Ala Asp Ser Pro Leu Val Lys Lys Ala Glu Gln

Ile	Asn	Gln	Ala 20	Gln	Gln	Asp	Ile	Gln 25	Thr	Ile	Thr	Pro	Ser 30	Gly	Leu
Asp	Ile	Pro 35	Ile	Val	Gly	Pro	Ser 40	Gly	Ser	Ala	Ala	Ser 45	Ala	Gly	Ser
Ala	Ala 50	Gly	Ala	Leu	Lys	Ser 55	Ser	Asn	Asn	Ser	Gly 60	Arg	Ile	Ser	Leu
Leu 65	Leu	Asp	Asp	Val	Asp 70	Asn	Glu	Met	Ala	Ala 75	Ile	Ala	Met	Gln	Gly 80
Phe	Arg	Ser	Met	Ile 85	Glu	Gln	Phe	Asn	Val 90	Asn	Asn	Pro	Ala	Thr 95	Ala
Lys	Glu	Leu	Gln 100	Ala	Met	Glu	Ala	Gln 105	Leu	Thr	Ala	Met	Ser 110	Asp	Gln
Leu	Val	Gly 115	Ala	Asp	Gly	Glu	Leu 120	Pro	Ala	Glu	Ile	Gln 125	Ala	Ile	Lys
Asp	Ala 130	Leu	Ala	Gln	Ala	Leu 135	Lys	Gln	Pro	Ser	Ala 140	Asp	Gly	Leu	Ala
Thr 145	Ala	Met	Gly	Gln	Val 150	Ala	Phe	Ala	Ala	Ala 155	Lys	Val	Gly	Gly	Gly 160
Ser	Ala	Gly	Thr	Ala 165	Gly	Thr	Val	Gln	Met 170	Asn	Val	Lys	Gln	Leu 175	Tyr
Lys	Thr	Ala	Phe 180		Ser	Thr	Ser	Ser 185		Ser	Tyr	Ala	Ala 190	Ala	Leu
Ser	Asp	Gly 195		Ser	Ala	Tyr	Lys 200	Thr	Leu	Asn	Ser	Leu 205		Ser	Glu
Ser	Arg 210		Gly	Val	Gln	Ser 215	Ala	Ile	Ser	Gln	Thr 220	Ala	Asn	Pro	Ala
Leu 225		Arg	Ser	Val	Ser 230		Ser	Gly	Ile	Glu 235		Gln	Gly	Arg	Ser 240
Ala	Asp	Ala	Ser	Gln 245	Arg	Ala	Ala	Glu	Thr 250		Val	Arg	Asp	Ser 255	Gln
Thr	Leu	Gly	Asp 260		Tyr	Ser	Arg	Leu 265		Val	Leu	Asp	Ser 270	Leu	Met
Ser	Thr	11e 275		Ser	Asn	Pro	Gln 280		Asn	Gln	Glu	Glu 285	Ile	Met	Glr
Lys	Leu		Ala	Ser	lle	Ser		Ala	Pro	Gln	Phe 300	Gly	Tyr	Pro	Ala

Val Gln Asn Ser Val Asp Ser Leu Gln Lys Phe Ala Ala Gln Leu Glu 305 310 315 320

Ārg Glu Phe Val Asp Gly Glu Arg Ser Leu Ala Glu Ser Gln Glu Asn 325 330 335

Ala Phe Arg Lys Gln Pro Ala Phe Ile Gln Gln Val Leu Val Asn Ile 340 345 350

Ala Ser Leu Phe Ser Gly Tyr Leu Ser 355 360

<210> 300

<211> 207

<212> PRT

<213> Chlamydia

<400> 300

Ser Ser Lys Ile Val Ser Leu Cys Glu Gly Ala Val Ala Asp Ala Arg
5 10 15

Met Cys Lys Ala Glu Leu Ile Lys Lys Glu Ala Asp Ala Tyr Leu Phe 20 25 30

Cys Glu Lys Ser Gly Ile Tyr Leu Thr Lys Lys Glu Gly Ile Leu 1le 35 40 45

Pro Ser Ala Gly Ile Asp Glu Ser Asn Thr Asp Gln Pro Phe Val Leu 50 55 60

Tyr Pro Lys Asp Ile Leu Gly Ser Cys Asn Arg Ile Gly Glu Trp Leu 65 70 75 80

Arg Asn Tyr Phe Arg Val Lys Glu Leu Gly Val Ile Ile Thr Asp Ser 85 90 95

His Thr Thr Pro Met Arg Arg Gly Val Leu Gly Ile Gly Leu Cys Trp 100 105 110

Tyr Gly Phe Ser Pro Leu His Asn Tyr Ile Gly Ser Leu Asp Cys Phe 115 120 125

Gly Arg Pro Leu Gln Met Thr Gln Ser Asn Leu Val Asp Ala Leu Ala 130 135 140

Val Ala Ala Val Val Cys Met Gly Glu Gly Asn Glu Gln Thr Pro Leu 145 150 155 160

Ala Val Ile Glu Gln Ala Pro Asn Met Val Tyr His Ser Tyr Pro Thr 165 170 175

Ser Arg Glu Glu Tyr Cys Ser Leu Arg Ile Asp Glu Thr Glu Asp Leu 180 185 190

શામકોન કાઈએ કારોન્ટર ત્યારો હતા. તાલ્યકાર સારીઓ રહેટો ઉપાય કાર ઉદ્યોગી કિંકો કોરોની કરિકો કોઈસો કોઇસ્ટર કે વહાઈ સો ઉ

Tyr Gly Pro Phe Leu Gln Ala Val Thr Trp Ser Gln Glu Lys Lys 195 200 205

<210> 301

<211> 183

<212> PRT

<213> Chlamydia

<400> 301

Ile Pro Pro Ala Pro Arg Gly His Pro Gln Ile Glu Val Thr Phe Asp
5 10 15

Ile Asp Ala Asn Gly Ile Leu His Val Ser Ala Lys Asp Ala Ala Ser 20 25 30

Gly Arg Glu Gln Lys Ile Arg Ile Glu Ala Ser Ser Gly Leu Lys Glu
35 40 45

Asp Glu Ile Gln Gln Met Ile Arg Asp Ala Glu Leu His Lys Glu Glu
50 55 60

Asp Lys Gln Arg Lys Glu Ala Ser Asp Val Lys Asn Glu Ala Asp Gly 65 70 75 80

Met Ile Phe Arg Ala Glu Lys Ala Val Lys Asp Tyr His Asp Lys Ile 85 90 95

Pro Ala Glu Leu Val Lys Glu Ile Glu Glu His Ile Glu Lys Val Arg 100 105 110

Gln Ala Ile Lys Glu Asp Ala Ser Thr Thr Ala Ile Lys Ala Ala Ser 115 120 125

Asp Glu Leu Ser Thr Arg Met Gln Lys Ile Gly Glu Ala Met Gln Ala 130 135 140

Gln Ser Ala Ser Ala Ala Ala Ser Ser Ala Ala Asn Ala Gln Gly Gly 145 150 155 160

Pro Asn Ile Asn Ser Glu Asp Leu Lys Lys His Ser Phe Ser Thr Arg 165 170 175

Pro Pro Ala Gly Gly Ser Ala 180

<210> 302

<211> 232

<212> PRT

<213> Chlamydia

<400> 302

Met Thr Lys His Gly Lys Arg Ile Arg Gly Ile Gln Glu Thr Tyr Asp

Mitsilikkaihiktiksiitejjästikkkiin ja sastan ja manna propertion aipestein inskinimi omaliamikse, ohistonakain

Leu Ala Lys Ser Tyr Ser Leu Gly Glu Ala Ile Asp Ile Leu Lys Gln 20 25 30

Cys Pro Thr Val Arg Phe Asp Gln Thr Val Asp Val Ser Val Lys Leu 35 40 45

Gly Ilé Asp Pro Arg Lys Ser Asp Gln Gln Ile Arg Gly Ser Val Ser 50 60

Leu Pro His Gly Thr Gly Lys Val Leu Arg Ile Leu Val Phe Ala Ala 65 70 . 75 80

Gly Asp Lys Ala Ala Glu Ala Ile Glu Ala Gly Ala Asp Phe Val Gly 85 90 95

Ser Asp Asp Leu Val Glu Lys Ile Lys Gly Gly Trp Val Asp Phe Asp 100 105 110

Val Ala Val Ala Thr Pro Asp Met Met Arg Glu Val Gly Lys Leu Gly 115 120 125

Lys Val Leu Gly Pro Arg Asn Leu Met Pro Thr Pro Lys Ala Gly Thr 130 135 140

Val Thr Thr Asp Val Val Lys Thr Ile Ala Glu Leu Arg Lys Gly Lys 145 150 155 160

Ile Glu Phe Lys Ala Asp Arg Ala Gly Val Cys Asn Val Gly Val Ala 165 170 175

Lys Leu Ser Phe Asp Ser Ala Gln Ile Lys Glu Asn Val Glu Ala Leu 180 185 190

Cys Ala Ala Leu Val Lys Ala Lys Pro Ala Thr Ala Lys Gly Gln Tyr 195 200 205

Leu Val Asn Phe Thr Ile Ser Ser Thr Met Gly Pro Gly Val Thr Val 210 215 220

Asp Thr Arg Glu Leu Ile Ala Leu 225 230

<210> 303

<211> 238

<212> PRT

<213> chlamydia

<400> 303

Ile Asn Ser Lys Leu Glu Thr Lys Asn Leu Ile Tyr Leu Lys Leu Lys

Ide Lys Lys Ser Phe Lys Met Gly Asn Ser Gly Phe Tyr Leu Tyr Asn 20 25 30

Thr Gln Asn Cys Val Phe Ala Asp Asn Ile Lys Val Gly Gln Met Thr Glu Pro Leu Lys Asp Gln Gln Ile Ile Leu Gly Thr Thr Ser Thr Pro 55 Val Ala Ala Lys Met Thr Ala Ser Asp Gly Ile Ser Leu Thr Val Ser Asn Asn Pro Ser Thr Asn Ala Ser Ile Thr Ile Gly Leu Asp Ala Glu Lys Ala Tyr Gln Leu Ile Leu Glu Lys Leu Gly Asp Gln Ile Leu Gly Gly Ile Ala Asp Thr Ile Val Asp Ser Thr Val Gln Asp Ile Leu Asp Lys Ile Thr Thr Asp Pro Ser Leu Gly Leu Leu Lys Ala Phe Asn Asn 135 Phe Pro Ile Thr Asn Lys Ile Gln Cys Asn Gly Leu Phe Thr Pro Arg Asn Ile Glu Thr Leu Leu Gly Gly Thr Glu Ile Gly Lys Phe Thr Val 165 Thr Pro Lys Ser Ser Gly Ser Met Phe Leu Val Ser Ala Asp Ile Ile 185 Ala Ser Arg Met Glu Gly Gly Val Val Leu Ala Leu Val Arg Glu Gly Asp Ser Lys Pro Tyr Ala Ile Ser Tyr Gly Tyr Ser Ser Gly Val Pro 215 Asn Leu Cys Ser Leu Arg Thr Arg Ile Ile Asn Thr Gly Leu

rallotstkartasikin ett orstorde ambilgapouri ombasi on gotom omtanknja teldiklojih katalihlah i silikilish katanindi distari lidlikh attahidah